OXIDATION OF CARBON AND NITROGEN IN THE ROOT-ZONE OF EMERGENT MACROPHYTES GROWN IN WETLAND MICROCOSMS

By

PETER SMILEY BURGOON

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At times one must bend until surely one will break . . . but instead of breaking something profoundly new and unexpected manifests. Fears, blinding illusions, disappear in the light of new insights. Life becomes not just bending, but surrendering, dying, and being reborn. Hail! the joys and pains of life and death.

P. S. Burgoon

True end is not the reaching of the limit,
but in a completion that is limitless.

Rabindranath Tagore

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OXIDATION OF CARBON AND NITROGEN IN THE ROOT-ZONE OF EMERGENT MACROPHYTES GROWN IN WETLAND MICROCOSMS

By

Peter Smiley Burgoon

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Use of constructed wetlands designed for wastewater treatment is becoming common throughout the world. Oxidation of carbon (C) and nitrogen (N) may be increased by transport of oxygen (O_2) into the rhizosphere of aquatic plants and periodic draining of the wetland. Field studies were conducted to determine: i) the influence of plants and draining of batch-load vegetated submerged beds (VSBs) on the oxidation of C and N from wastewater, ii) the effect of hydraulic retention time (HRT) in VSBs on first-order CBOD removal rate coefficients and, iii) the efficiency of C removal in VSBs and floating aquatic macrophyte systems (FAMs). Plants had significant effects on C and N oxidation, however, after an 18 hour HRT there was no difference between VSBs with and without plants. Draining

VSBs had no effect on oxidation of C and N. Greater than 90% CBOD removal occurred within 18 hours. The first-order CBOD removal rate model was not appropriate after 24 hours. At a hydraulic loading rate of 20 cm/day, VSBs removed more CBOD than FAMs. Oxygen transport in VSBs estimated from C oxidation was 28.6 g/m^2 day, and for N oxidation 2.4 g/m^2 day. These rates agree with published O_2 transport rates.

Laboratory studies evaluated C and N oxidation in the rhizopshere of plants grown in wetland microcosms. Nitrate- and sulfate-reducing biofilms were established on plastic matrix in anaerobic continuous-flow stirred tank reactors with and without *Scirpus validus*. Steady state pH and Eh were maintained for at least 40 days in reactors. Redox potential was not affected by the presence of plants. An acetate mass balance accounted for reduction of NO_3^-, SO_4^{-2}, and Fe^{+3}, and methanogenesis. Differences in acetate mass balance between reactors with and without plants, were used to estimate oxygen transport through the plants. Oxygen transport rate estimated from acetate mass balance was about 10 g O_2/m^2 day; and the estimated rate from $^{15}NH_4$ oxidation was 0.5 g O_2/m^2 day. This reactor design for simulating anaerobic wetland environments may be a useful tool for studying plant/microbial interactions, and for studying treatment of wastewater in wetlands.

CHAPTER 1
INTRODUCTION

Use of constructed and natural wetlands for wastewater treatment has increased immensely in the last decade. Constructed wetlands are being used for municipal, agricultural, and industrial waste treatment in the United States (Reed and Brown 1992; Conley et al. 1991) and around the world (Reddy and Smith 1987; Hammer 1989; Cooper and Findlater 1990; Cooper 1990; IAWQ 1992). These systems represent an inexpensive yet effective method for treating waste in rural environments or for ecosystem restoration. The types of designs and waste treatment systems are diverse. Successful, and experimental operations are scattered across the country. A few examples include: (1) use in coal mining areas where impacts of acid mine drainage with high concentrations of sulfate have been ameliorated with wetlands (Kleinmann and Girts 1987); (2) municipal waste treatment in the estuaries of Humbolt Bay, California (Gearheart et al. 1989); (3) septage waste treatment in artificial wetland ecosystems in a greenhouse on Cape Cod (Teal and Peterson 1993); and (4) a 1500 acre marsh being built for the restoration of Lake Apopka in Florida (Lowe et al. 1989). Constructed wetlands may be designed to provide secondary, advanced or tertiary waste treatment (Reed et al. 1988). Accompanying benefits are enhanced wildlife habitat, flood amelioration and aesthetic beauty.

There are two basic types of constructed wetlands: free- water surface systems (FWS), with flow of water over the surface of the wetland, and subsurface flow, in which water flows laterally through the root matrix. Vegetated Submerged Bed (VSB) refers to subsurface flow wetlands (Reed et al. 1988), this terminology will be used throughout this dissertation. In Europe and the United States, VSBs have been a popular treatment option for small community waste treatment (Cooper and Findlater 1990). More than 90% of the VSBs in the United States treat less than 3800 m^3/d (1 mgd); the FWS wetlands receive larger flows, up to 76000 m^3/day (20 mgd) (Reed and Brown 1992).

Statement of the Problem

A decade of research and operational experience have provided a foundation for development of engineering design manuals in the United States (Reed et al. 1988; USEPA 1988; WPCF 1990) and Europe (Cooper 1990). However, in a current review of constructed wetlands in the United States, Reed and Brown (1992) note that for FWS and VSB wetlands, "the design approach . . . ranges from 'seat of the pants' empirical to more rational models from a limited data base"(p 778). Although design information is available for

BOD and nitrogen (N) removal, basic processes for removal of carbon (C) and N are poorly understood.

Due to limited information on removal processes, design engineers choose the most conservative approach to ensure adequate treatment. This eliminates data from systems that have above average performance, and which may actually represent the true capacity of a wetland system to remove pollutants. For example, Reed et al. (1988) suggest use of first-order BOD removal rate coefficients which are an order of magnitude smaller than reported coefficients (Burgoon et al. 1991; Conley et al. 1991). Further work is necessary to understand and optimize rate constants and other relationships that govern system performance (Reed and Brown 1992). Reed and Brown (1992) note that design models for N removal are based on "lumped data from FWS and VSBs and may be useful only as a first approximation"(p 779). Increased understanding of biochemical and physico-chemical processes functioning in the system will enhance design optimization and ensure both economic and environmental success of a constructed wetland.

Need for Research

Processes active in wetlands used for wastewater may be generalized as physical, chemical and biological (Table 1.1). Physical processes have dominant effects in systems receiving wastewater high in solids, such as

Table 1.1 Basic processes involved in constructed wetlands used for wastewater treatment.

General processes	Specific processes	Waste component removed
Physical	1. straining 2. sedimentation	1. suspended solids (inert and biodegradable) 2. organic C, N and P.
Chemical	1. adsorption 2. precipitation	1. Dissolved organic compounds 2. Anions, PO_4^{4-} 3. Cations, Fe^{+2}, Fe^{+3}, Ca^{+2},
Biological		
a. Microbial	1. Oxidation of C 2. Oxidation and reduction of N, Mn, Fe, and S 3. Assimilation of C, N and P.	1. particulate and soluble organic matter 2. NH_4^+, NO_2^-, NO_3^-, SO_4^{2-}, Fe^{+3}, PO_4^{2-} 3. Chlorinated hydrocarbons, aromatics, pesticides and xenobiotics
b. Plants	1. Plant growth 2. Transport of O_2 into the rhizosphere 3. Release organic matter to be used as electron donor.	1. NH_4^+, NO_3^- 2. PO_4^{2-} 3. Macro and micronutrients 4. organic matter

settled wastewater. In systems used for advanced and tertiary wastewater treatment soluble forms of C and N and phosphorus (P) are the pollutants of concern. These are most affected by chemical and biological removal processes.

Plants are the most conspicuous component of wetlands. A wide variety of herbaceous and woody plants permeate the natural wetland landscape. Polycultures of species are common in FWS wetlands, whereas monocultures of a few specific species are used in VSBs. In Europe, the plant of choice has been the cold tolerant, common reed, Phragmites australis. Although experiments by Gersberg et al. (1983, 1986), have shown Scirpus validus to be superior to P. australis and Typha latifolia for C and N removal in VSBs, all three plants are commonly used in VSBs around the world.

Plants assimilate nutrients into above- and below-ground biomass, with as much as 33% of removed N stored in root biomass (Rogers et al. 1991). Young growing plants may assimilate up to 85% of wastewater N (Rogers et al. 1991). However, full-sized treatment systems must operate with mature plants, consequently nutrient uptake is significantly reduced. Generally, harvesting plant biomass is not a practical method for N removal (Crites and Tchobanoglous 1992).

The primary function of plants in constructed wetlands is to transport O_2 into the rhizosphere. Gersberg et al. (1986, 1983) found that VSBs with plants removed significantly more N than without plants. Many species are able to oxygenate their roots (Armstrong 1967; Armstrong and Armstrong 1988; Grosse 1989; Moorhead and Reddy 1988) however the ability is highly variable and well studied in only one species, Phragmites australis (Armstrong and Armstrong 1990a,b; Armstrong et al. 1988; 1990). The capacity for O_2 transport into the rhizosphere is well documented (Armstrong and Armstrong 1990a; Moorhead and Reddy 1988; Grosse 1988; Dacey 1980). However, Brix (1990) has shown that no O_2 leaks into the roots of P. australis grown in a constructed wetland. Specific rates of O_2 transport which may be used in a rational design process are not available (Reed and Brown 1992; Cooper 1990).

The major source of O_2 in the FWS wetlands is from reaeration at the exposed water surface (Reed and Brown 1992). Oxygen transport is considered less critical in FWS wetlands because water passes over the surface of the wetland. The major source of O_2 for treatment in VSBs is through the plants (Reed and Brown 1992). A fundamental design consideration is the amount of O_2 that leaks out of the roots, and is available to the aerobic hetero- and autotrophic microbes associated with the roots.

Environmental factors influencing O_2 transport in P. australis are sunlight, humidity, and wind. Thermo-osmotic pressure differentials induced by sunlight have been found to be the major driving force of convective flows of air into the roots of many emergent aquatic plants (Grosse 1989; Armstrong and Armstrong 1990b). This mechanism provides O_2 for root respiration. However, Mendelssohn et al. (1981) and McKee et al. (1989) found that root respiration is dependent on the oxidation-reduction potential of the soil. This implies that O_2 transport may not satisfy the respiratory demand of roots if conditions are highly reduced. If this is so, then wetlands may need to be designed to encourage specific redox environments for optimal treatment.

Objectives

The overall objective of this dissertation is to estimate O_2 transport through an emergent aquatic plant based on the oxidation of C and N in the root zone. To evaluate the role of O_2 transport in wastewater treatment, field experiments were designed to address the following questions:

(i) How do plants affect removal of C and N in VSBs that receive primary and secondary effluent?

(ii) Does draining the VSB introduce enough air to improve C and N oxidation in a VSB? is it more effective than plants?

(iii) What are estimated O_2 transport rates through plants based on oxidation of C and N ?

Objectives in experiments designed to address these questions were

(i) Operate VSBs in batch-load and continuous modes to determine the effect of draining and filling on bringing air into the rhizosphere.

(ii) Determine the first-order removal rates of CBOD in the VSBs.

(iii) Estimate O_2 transport based on removal of C and N in the VSBs.

Laboratory experiments were designed to examine the effects of anaerobiosis on O_2 transport under controlled environmental conditions. Questions posed were

(i) Is O_2 transport through plants affected by the anaerobic condition of the rhizosphere?

(ii) Can O_2 transport through plants grown in anaerobic environments be accurately estimated from C and N oxidation?

The objectives in the experiments designed to address these questions were

(i) Design an anaerobic biofilm reactor with steady state Eh and pH which simulates anaerobic wetland environments.

(ii) Establish conditions in the reactors which are conducive to growth of an emergent aquatic plant.

(iii) Estimate O_2 transport by Scirpus validus based on oxidation of C and N in anaerobic environments.

Laboratory and field studies were based on the following hypotheses: (i) Plants transport O_2 into the root zone in excess of root respiratory requirements; (ii) excess O_2 is available to aerobic hetero- and autotrophic bacteria; (iii) O_2 transport is independent of the oxidation-reduction environment of the rhizosphere.

Dissertation Format

The progression of this research moves from field studies to the use of laboratory microcosms for a better understanding the results observed in field-scale systems. Chapter 2 presents field studies using VSBs to remove C and N from primary and secondary wastewater. Chapters 3 and 4 present the laboratory studies. Chapter 3 consists of two sections, the first covers reactor construction, and evaluation of complete-mix and steady state conditions in the reactors. The second section present results of establishing the anaerobic biofilms, and reactor chemistry. Chapter 4 presents results of estimating O_2 transport through the S. validus planted in the reactors. Oxygen transport was based on (i) mass balance of C in reactors with and without plants and; (ii) oxidation of $^{15}NH_4$. Chapter 5 is a summary of the dissertation followed by a discussion of the usefulness of the research for studying contemporary environmental problems.

CHAPTER 2
PERFORMANCE OF VEGETATED SUBMERGED BEDS UNDER BATCH-LOADING AND CONTINUOUS-FLOW CONDITIONS

Introduction

Constructed wetlands have become a popular waste treatment alternative for small communities in the United States and Europe (Cooper and Findlater 1991; Hammer 1989; Reddy and Smith 1987). Ideally, they offer effective treatment with low initial capital and operational costs. A vegetated submerged bed (VSB) is a type of constructed wetland in which wastewater flows through the plant rooting media (via sub-surface flow) instead of over the surface of the wetland. Numerous studies have verified the effectiveness of VSBs in treating wastewater (Gersberg et al. 1983; Hammer 1989; Cooper and Findlater 1990) and have provided a data base for the development of design manuals (Reed et al. 1988; USEPA 1988; Cooper 1990). Design criteria developed for these systems have been based on changes in concentration of selected parameters such as biological oxygen demand (BOD), nitrogen (N), and phosphorus (P), with little attention to processes functioning in the wetland. The design information for carbon (C) and N removal is further restricted by conflicting experimental data from batch and continuous flow systems (Burgoon et al. 1991). Basic understanding of the processes functioning within VSBs that account for removal of C and N is critical for optimizing VSBs to attain maximum treatment efficiency.

Pilot-scale studies with batch-load VSBs provide a simple, rapid method for obtaining performance data which can be used to design wetlands for wastewater treatment. However, batch-load systems have yielded first-order BOD removal rate coefficients that are significantly larger than those obtained for field-scale continuous-flow VSBs (Wolverton et al. 1983; Reed et al.1988; Burgoon et al. 1991). Although BOD removal in VSBs is assumed to follow first-order kinetics, the validity of this assumption has not been demonstrated.

Alternate draining and flooding of the VSB during batch-loading may result in aeration of porewater and biofilms within the soil and gravel matrix (entrainment) (Seidel 1976; Burgoon 1989; Brix and Schierup 1990). Carbon and N oxidation and removal may be improved if significant amounts of O_2 are available to the microbiota after draining and refilling of the VSB with wastewater. Availability of air will increase if enough O_2 diffuses into the biofilm to significantly increase oxidation of C and N by heterotrophic and autotrophic bacteria. Diffusion of O_2 into biofilms in trickling filters has been quantified with O_2 microelectrodes (Bungay et al. 1969; Kuenen et al. 1986).

The amount of O_2 saturating the biofilm depends on the specific surface area of the gravel/soil matrix and plant roots, depth of the biofilm, microbial metabolism (Kuenen et al. 1986), and length of time that the VSB is drained.

Active transport of O_2 into the root zone through plants was shown to have significant effects on oxidation of C and N in ponds and wetlands (Reddy et al. 1990; Moorhead and Reddy 1988; Gersberg et al. 1983; Wolverton et al. 1983). Howes et al. (1984) concluded that mass flow of air into the soil matrix during tidal excursions in an estuary resulted in significantly greater oxidation of organic matter than would occur due to transport of O_2 through emergent macrophytes. The relative importance of O_2 transport through plants versus entrainment of air in VSBs operated in batch-load has not been evaluated.

The objectives of this study are to determine: (1) the effects of emergent aquatic plants on the oxidation of C and N in batch-load VSBs and, (2) the influence of hydraulic retention time (HRT) on first-order removal rate coefficients for continuous and batch-load VSBs. The hypothesis being tested was that batch-load operation (vs continuous flow) of a VSB will augment the oxidation of C and N. If entrainment of O_2 (due to drain and fill operation) is an effective aeration mechanism in VSBs, a batch-load VSB should remove more C and N than a continuous flow VSB.

Materials and Methods

The constructed wetland systems were set-up outdoors, at the Reedy Creek Improvement District Wastewater Treatment Plant located in Lake Buena Vista, Florida. Description of experiments are as follows:

Experiment I. Batch-load VSBs

This experiment was designed to test the hypothesis that (i) batch-load operation of a VSB will introduce air into the root zone and consequently increase the rate of oxidation of C and N, and (ii) first-order BOD removal rate coefficients are different for batch and continuous flow VSBs.

Operation and experimental setup

The VSBs (6 x 1 x 0.4 m) contained river gravel (diameter = 3 cm, porosity = 28%, specific surface area = 100 m^2/m^3) as the rooting medium. The six VSBs used in this study had previously received a continuous flow of primary settled wastewater (CBOD = 220 mg/L, SS = 70 mg/L) or secondary effluent (CBOD = 15 mg/L, NH_4-N = 25 mg/L) from water hyacinth (*Eichhornia crassipes* [Mort] solm) ponds for two years (November 1987 to December 1989) prior to beginning these experiments. The emergent macrophyte, *Scirpus pungens* L. (swordstem bulrush) was established during a two year period in the VSBs. Prior to initiation of this experiment, dead plant biomass was removed from the gravel surface. Four of the six VSBs were plumbed for batch-loading of wastewater (Figure 2.1). Two of the batch-load VSBs were left nonvegetated (NVSB) to serve as controls. A standpipe on the outside of the batch-load VSB was used to set the water level at 35 cm in the batch-load VSBs. A #14 rubber stopper at the bottom of the pipe was used to drain the VSBs at regular intervals. Batch-load VSBs were operated for six weeks before experimental data was collected. Batch-load VSBs which received primary wastewater were drained and refilled after a three day retention time; VSBs which received secondary effluent were drained and refilled after six days. Two of the six VSBs remained in operation as continuous flow VSBs. One received primary wastewater at hydraulic loading rates of 10 cm/day (January 1, 1990 to June 1 1990) and 20 cm/day (June 1 to July 15, 1990); the other received secondary wastewater at a hydraulic loading of 10 cm/day. Use of two hydraulic loading rates of settled sewage allowed comparison of CBOD removal rate coefficients from the batch and continuous-flow VSBs at 0.5 and 1.0 day hydraulic retention times (20 and 10 cm/day hydraulic loading rates respectively).

At each sampling time, water loss due to evapotranspiration (ET) was measured by monitoring the water level changes in the stand pipe. Experiments in VSBs with 3 day HRTs were terminated if rainfall occurred within the 3

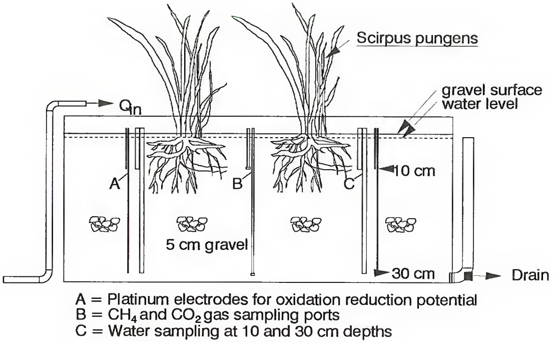
Scirpus pungens
gravel surface
water level
Q_{in}
A
B
C
10 cm
30 cm
5 cm gravel
Drain
A = Platinum electrodes for oxidation reduction potential
B = CH_4 and CO_2 gas sampling ports
C = Water sampling at 10 and 30 cm depths

Figure 2.1. Vegetated submerged bed plumbed to receive batch-loads of primary or secondary wastewater effluent. Placement of Eh electrodes and tubes for collecting water and gas samples are shown. Water level was kept 2-3 cm below the top surface of the gravel.

day HRT. Experiments on VSBs with 6 day HRTs were terminated when rainfall caused the VSB to flood.

When these experiments were initiated, the root mass of the Scirpus pungens extended 10 to 15 cm deep into the gravel bed. Standing crop biomass in each VSB was determined by complete harvest of the VSBs at the end of the experiment.

Water sampling and analysis

All VSBs and NVSBs had sampling tubes (1.25 cm PVC) set at two depths, one in the root zone (10 cm) and the other below the root zone (30 cm) (Figure 2.1). Water samples were collected from March 1 to July 15, 1990, for the batch-load and continuous-flow VSBs. Influent samples were taken as the VSBs filled with wastewater. Batch-load VSBs receiving primary settled wastewater were sampled at t = 6, 12, 24, 36, 48, and 72 hours; batch-load VSBs receiving secondary effluent were sampled once a day for six days. A hand-held suction pump was used to draw samples from the 10 and 30 cm sampling tubes. After several tube volumes had been displaced (about 200 mL), a water sample was collected and analyzed for CBOD[1] (method 507, APHA 1989) within one half hour. Water samples were filtered through glass fiber filters (1 μm), frozen and analyzed within one month for

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[1] CBOD = oxygen demand due to heterotrophic oxidation of organic matter, a nitrification inhibitor is added to stop O_2 demand of nitrifying bacteria.

NH_4-N and $(NO_3 + NO_2)$-N using the automated salicylate method (method 361.2 EPA 1983) and the automated cadmium reduction method (method 418 C, APHA 1989) respectively. All EPA check samples used to confirm methods for analyzing CBOD, NH_4-N and $(NO_3 + NO_2)$-N were within the accepted 95% confidence limits.

Suspended solids (SS) and flowrate of the effluent were measured at regular intervals throughout the 15 minute drain time. Flow rates were measured using a graduated cylinder and stop watch. Suspended solids samples were analyzed according to method 2540 D (APHA 1989). Concentrations of SS varied during drainage; therefore, flow weighted concentrations were calculated.

After four sets of CBOD removal data were obtained from VSBs receiving primary effluent, soluble CBOD removal rates were evaluated. Influent wastewater and pore water samples were filtered through 0.45 μm (used as a pre-filter) and 0.2 μm millipore filters for removal of particulate organic matter. Bacteria are removed by filtration through the 0.2 μm filter; therefore, an inoculant was used in the CBOD analysis. Inoculant was prepared using 5 mL of primary sewage effluent (filtered through a glass fiber filter) diluted in 1 L of deionized water. One milliliter of this solution was used to inoculate the 300 mL CBOD bottles. Loss of dissolved O_2 in CBOD bottles with 1 mL of inoculant was less than 0.5 mg/L. Analysis of soluble CBOD enabled estimation of the CBOD removed by sedimentation and straining of organic material within the gravel matrix. If sedimentation is a primary mechanism for CBOD removal we expect no change in soluble CBOD over the three day HRT.

First-order removal rate coefficients were calculated using Equation [2-1]. Coefficients were calculated, then converted to a coefficient expected at 20°c using Equation [2-2] (Reed et al. 1988)

$$k = -\frac{\ln (C_f/C_i)}{t} \qquad [2\text{-}1]$$

C_f = effluent CBOD, mg/L
C_i = influent CBOD, mg/L
t = treatment time within the VSB, days

$$k_{20} = \frac{k_T}{(1.06^{(T-20)})} \qquad [2\text{-}2]$$

T = water temperature within the VSB, °c

k_T = first-order removal rate coefficient at T °c.

Physico-chemical measurements

Platinum electrodes for measurement of oxidation-reduction potentials (Eh) were placed at 10 and 30 cm depths in the VSBs. Electrodes consisted of a 1 cm piece of platinum wire soldered to 16 gauge copper wire and a reference calomel electrode (Faulkner et al. 1989). All data are reported relative to a standard hydrogen half cell ($Eh_{measured}$ + 242 mV). Influent wastewater flowed through a 2.5 cm orifice as it entered the batch-load VSB and NVSB. The steady stream of water splashed on the gravel as the tanks were filled. Dissolved O_2 of the influent wastewater was measured by placing a YSI O_2 probe in a pool of water below the NVSB inlet as primary sewage effluent splashed into the NVSB.

Concentrations of SO_4-S and Fe in the influent wastewater were analyzed since they may also be used as electron acceptors in the oxidation of organic matter. Water samples collected during two of the CBOD studies were filtered through 1 μm glass fiber filters, frozen, then analyzed for SO_4-S within two weeks on a Dionex Ion Chromatograph (method 4110, APHA 1989). Iron was a concern because $FeCl_3$ was added to the raw sewage as it entered the waste treatment plant. Therefore, primary wastewater used in the VSBs had a high Fe content (10 - 20 mg/L total Fe). Oxidized and reduced forms of Fe in the influent were analyzed using the Phenanthroline method (3500-F3 D, APHA 1989). Oxidized and reduced forms of Fe were also measured immediately after the influent wastewater had splashed into the VSB to determine changes in oxidation of Fe^{+2} due to aeration. Iron measurements were made two times, both after the CBOD studies were finished.

Methane generation

Two ports for sampling dissolved gases (Figure 2.1) were placed at 10 and 30 cm depths in the batch-load VSBs (Faulkner et al. 1989). The air space within the gas sampling ports was purged with helium gas immediately after the VSB was filled with wastewater. After 12 or 24 hours about 8 mL of gas sample was pulled from the ports; 5 ml of each sample was stored in a vaccutainer. After each gas sampling, the ports were again purged with helium and sampled after 12 or 24 hours, for up to 72 hours. Gas samples were analyzed for CH_4 using a Hewlett-Packard 5840A gas chromatograph equipped with a 1.8 m x 2 mm I.D. stainless steel column packed with porpak Q (80 -100 mesh) and a thermal conductivity detector. Carrier gas (hydrogen) flow-rate of 10 mL/min, injector temperature of 100°C, oven temperature of 40°C and the detector temperature of 100°C were used.

Experiment II. Continuous-flow VSBs and FAMs

This experiment tests the hypothesis that (i) C and N removal in VSBs is not affected by the specific surface area or porosity of the rooting matrix, (ii) longer hydraulic retention times in floating aquatic macrophyte ponds (FAMs) will improve C and N removal relative to a VSB, and (iii) accumulation of solids in a VSB can be related to changes in hydraulic characteristic curves.

Experimental setup

Experiment IIa. Four 6 m^2 (6 x 1 x 0.4 m) VSBs were used for comparison of two different rooting matrices. Two tanks were filled with 5 cm river gravel (same as experiment

I) and two were filled with 5 cm Nor-pak plastic packing[2] (porosity = 90%, specific surface area 145 m^2/m^3). One set of two VSBs (1 gravel and 1 plastic rooting matrix) received a continuous flow of primary settled wastewater, while the other two VSBs received secondary effluent. Hydraulic loading rate of primary and secondary effluent was 10 cm/day. Hydraulic retention times in gravel and plastic filled VSBs were 24 and 80 hours respectively. These tanks were operated from November 1987 to December 1989.

Experiment IIb. This experiment compared treatment of primary effluent in two 6 m^2 tanks planted with the floating aquatic macrophyte, pennywort (Hydrocotyle umbellata) to treatment in three 6 m^2 VSBs (5 cm gravel) planted with the common arrowhead, Sagittaria latifolia (two tanks), and Scirpus pungens (one tank). Hydraulic loading rate of the wastewater was 20 cm/day for a 1.5 month period (June 1, 1990, and July 15, 1990); hydraulic retention time was 12 hours. All five VSBs and FAMs previously received primary wastewater (10 cm/day) for a two year period. Results for these experimental systems operated with a hydraulic loading rate of 10 cm/day have been reported previously in DeBusk et al. (1990).

Seedlings of Sagittaria latifolia and Scirpus pungens were planted (11 plants/m^2) during initial startup of the

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[2] Donated by Jeager Inc., Spring, Texas.

VSBs (November 1987). Tanks without rooting matrix (FAMs) were stocked with *Hydrocotyle umbellata*. Periodic application of insecticide was needed to control aphids on the *Sagittaria* and *Hydrocotyle*.

Water sampling and analysis

Influent and effluent water samples for Experiment IIa were collected weekly for two years (November 1987-November 1989) and analyzed for BOD (method 507, APHA 1989), NH_4-N, and (NO_2 + NO_3)-N. Analytical methods for NH_4-N and (NO_2 + NO_3)-N were the same as used in Experiment I. When the hydraulic load was increased to 20 cm/day in Experiment IIb, influent and effluent samples were analyzed for CBOD (method 507, APHA 1985). Analysis of samples with and without nitrification inhibitor (CBOD and BOD respectively) revealed that BOD results were affected by nitrification in the BOD bottles. A CBOD/BOD conversion ratio was calculated (based on comparison of samples evaluated for BOD and CBOD) so that BOD data from VSBs receiving 10 cm/day could be compared to CBOD data from VSBs receiving 20 cm/day.

Hydraulic characteristics

Fluorescent dye (Rhodamine WT) was used to characterize the hydraulic flow patterns within the gravel and plastic filled VSBs. Dye was added to the VSBs six months and one year after the VSBs were planted and began receiving wastewater effluent (10 cm/day). Fifty milliliters of dye (2400 mg/L) were added at the influent of the VSBs.

Effluent samples were collected at three hour intervals until the effluent fluorescence was less than 1 μg/L. Fluorescence was analyzed using a Turner Designs Fluorometer. Effluent concentration and sampling times were converted to their dimensionless equivalents using methods ("C" diagrams) from Middlebrooks et al. (1982). Pore volume of the VSBs were calculated once (one year after receiving wastewater) by measuring the time required to fill a VSB when water flowed in at a constant rate.

Nominal retention time of the dye within the VSB was calculated using the following equation:

$$\Theta_n = \frac{\int (C/C_o)\,\Theta d\Theta}{\int (C/C_o)\, d\Theta} \qquad [2\text{-}3]$$

Θ_n = nominal retention time (dimensionless)
Θ = dimensionless time (time of sampling divided by theoretical hydraulic retention time)
C = effluent dye concentration, mg/L
C_o = mass of dye added per tank pore volume, mg/L

Hypothesized changes in hydraulic patterns may be estimated using the nominal retention time as an indicator of clogging in the VSB. Theoretically, as the pores fill with solids the nominal retention time should decrease. Changes in nominal retention times should indicate changes in the hydraulic properties of the bed (due to accumulation of solids and/or root growth).

Statistical methods

Comparisons were made using the Statistical Analysis Systems version 6.04 (SAS 1987). A Generalized Linear Model t-test was used for comparison of treatment means, unless stated otherwise. All differences are reported as significant in a rejection region of $p \leq 0.05$.

Results

Experiment I. Batch-load VSBs

Temperature

Plants shaded the gravel and consequently moderated temperature variations of the batch-load VSBs. The largest variation in temperature recorded over a 72 hour period was from 22 to 30°C in the NVSB, and 23 to 27°C in the VSB. The mean temperatures within the VSBs and NVSBs during the batch-load studies were 25.0 ± 1.2 and 25.6 ± 2.1°C respectively. VSBs receiving a continuous-flow of wastewater also showed small variation in temperature due to shading by the plants. Mean temperatures within the continuous-flow VSBs and NVSBs were 26.3 ± 0.9 and 25.4 ± 0.6°C respectively.

Mean evapotranspiration (ET) was 0.9 cm/day. This is equivalent to a change in water depth of 3 cm/day. Evapotranspiration over a 6 day HRT resulted in as much as 45% water loss from the tank.

Table 2.1. Mean oxidation-reduction potentials in VSBs and NVSBs receiving primary and secondary wastewater. Measurements were made at 10 cm (in root zone) and 30 cm (below root zone).

	Oxidation-reduction potential							
Operational mode	Batch-load				Continuous-flow			
Wastewater type	primary		secondary		primary		secondary	
S. pungens	---------------------------- mV ----------------------------							
10 cm	-57 ± 212	a[+]	144 ± 199	a	-182 ± 96	a	-109 ± 156	a
30 cm	-229 ± 72	b	-172 ± 122	b	-215 ± 62	a	-97 ± 140	a
No plants								
10 cm	-217 ± 93	b	-171 ± 117	b	na	-	na	-
30 cm	-244 ± 26	b	-190 ± 109	b	na	-	na	-

[+] Comparison of means using a t-test for samples with unequal variance (Ott 1984). Different letters in a column are significantly different ($p \leq 0.05$).

na = treatment not available

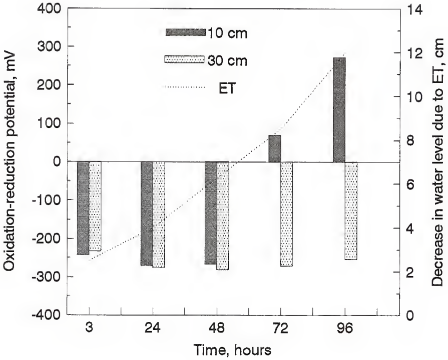


Figure 2.2. Redox potentials (Eh) and water levels at 10 and 30 cm depths within vegetated submerged beds filled with secondary wastewater effluent.

Oxidation-reduction potential (Eh)

The Eh of the VSBs was affected by water loss due to ET. High ET caused water levels to drop below the platinum electrode placed at the 10 cm depth, resulting in Eh values typical of oxidized conditions (Figure 2.2). These conditions resulted in high variability in Eh at the 10 cm depth in the VSB receiving primary and secondary effluent (Table 2.1). The Eh values were consistently less than -150 mV at the 30 cm depth in VSBs receiving primary or secondary effluent (Table 2.1). The Eh remained constant at the 30 cm depth (during the 3 day HRT) in batch-load VSBs and NVSBs despite greater than 95% removal of CBOD within one day.

The Eh in continuous-flow VSBs was less than -150 mV. Wastewater flow into the continuous-flow VSBs was greater than ET losses; therefore, the water level did not drop below the tip of the electrode placed 10 cm deep into the root zone. There was no difference in Eh at the influent and effluent end of the continuous-flow VSBs receiving primary or secondary effluent (data not shown). There was also no significant variation in Eh at 10 and 30 cm depths in the continuous flow VSB (Table 2.1).

CBOD removal in batch-load VSBs

After 6 and 12 hours, CBOD was significantly lower in the VSB than in the NVSB (Figure 2.3a). Within 6 hours, 78% of the CBOD (64 ± 4 g CBOD $/m^2$ day) in the VSB was removed compared to 55% (48 ± 12 g CBOD $/m^2$ day) in the NSVB. After

6 and 12 hours, CBOD was significantly lower in the VSB than in the NVSB (Figure 2.3a). However, within 18 hours the difference between the VSB and NVSB was not significant and CBOD had decreased from 220 mg/L to less than 20 mg/L in both VSBs and NVSBs. After 24 hours, greater than 95% of the CBOD was removed in both VSBs and NVSBs, with a mean removal of 21 ± 0.3 g CBOD /m^2 day (Figure 2.3b). These results suggest that plants improved CBOD removal in the VSBs only at HRTs less than or equal to 12 hours. There was no difference in CBOD removal between the samples taken at 10 (in root zone) and 30 cm (below root zone) depths.

Removal of soluble CBOD. Soluble CBOD of influent wastewater averaged 125 mg/L. Removal of soluble CBOD followed the same trends observed for total CBOD. After 6 hours, soluble CBOD concentration in the VSB was 33 mg/L, significantly lower than 66 mg/L, in the NVSB (Figure 2.3c). After 12 hours the soluble CBOD from the VSB and NVSB averaged 12 ± 2 mg/L. The rapid removal of soluble CBOD implies that VSBs do not operate simply as physical filters for straining and sedimentation of influent organic material.

First-order CBOD removal rate coefficients. First-order CBOD removal rate coefficients were similar for batch and continuous-flow VSBs with a HRT of 1 day (Table 2.2). The results show that CBOD is removed rapidly within the

Table 2.2 First-order CBOD removal rate coefficients for batch and continuous flow VSBs (calculated for T = 20°C, $k_{20} = k_t/(1.06\hat{}(T-20))$ (Reed et al. 1988). Values in parentheses are sample standard deviations.

	Hydraulic retention time, hours							
Treatment	6	n	12	n	18	n	24	n
	----------------- days^{-1} -----------------							
Batch-load								
S. pungens	4.4 (0.8)	9	2.9 (0.4)	9	2.1 (0.1)	9	1.7 (0.2)	9
No plants	2.2 (1.1)	9	2.8 (0.4)	9	2.7 (0.1)	9	2.4 (0.5)	9
Continuous								
S. pungens	na	na	5.0 (0.9)	24	na	na	2.0 (0.2)	6

n = number of data points
na = data not available

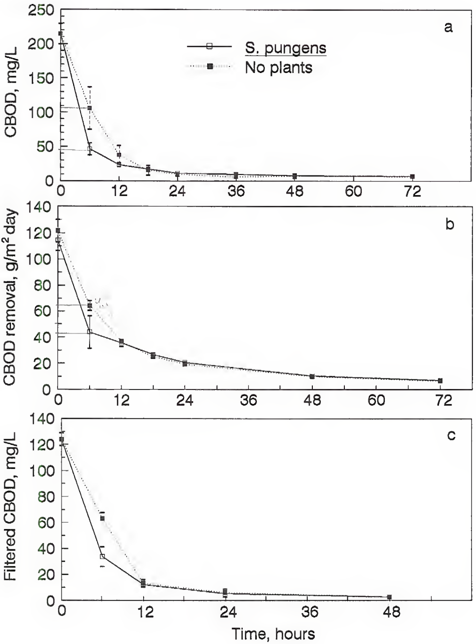


Figure 2.3 Carbonaceous biochemical oxygen demand (CBOD) measured at 30 cm depth in batch-load vegetated (Scirpus pungens) and nonvegetated submerged beds receiving primary wastewater. a) Effluent CBOD concentration; b) Mass removal of CBOD; c) Removal of soluble CBOD.

batch-load and continuous-flow VSBs (Figure 2.3); therefore, removal rate coefficients decreased as HRT increased (Table 2.2). Removal rate coefficients for continuous-flow VSBs were significantly larger than batch-load VSBs also operated at an HRT of 12 hours.

Effect of electron acceptors on CBOD removal. Electron acceptors (O_2, NO_3^-, Fe^{3+}, SO_4^{2-}) entering VSBs and NVSBs were measured to determine their relative contribution to oxidation of organic matter in the VSB. Dissolved O_2 content of the influent, after splashing into a NVSB was 2 mg/L. This was assumed as the O_2 concentration of influent entering VSBs and NVSBs. At the sewage treatment plant, $FeCl_3$ is added to the raw sewage prior to sedimentation in order to control odor and remove phosphorus. Analysis of primary sewage effluent entering VSBs and NVSBs showed that >98% of Fe was in reduced form (Fe^{+2}) as it entered VSBs and NVSBs. After splashing onto the gravel surface, approximately 13% of the Fe was oxidized. This is in agreement with an expected oxidation rate of Fe at pH = 6.8 in an aerated environment (Benefield et al. 1982). Nitrates were not detected in the influent; sulfates were less than 1.5 mg/L. Electron acceptors (O_2, NO_3^-, Fe^{+3}, and SO_4^{-2}) entering the VSB via the influent were calculated to contribute to less than 2% of the total CBOD removal within VSBs. Therefore >98% of the CBOD was removed by a

Table 2.3 Methane production at 10 and 30 cm depths in vegetated submerged beds receiving primary and secondary wastewater effluent.

		Rate CH_4 increase			
		Primary		Secondary	
HRT, hours	Depth, cm	%[+] CH_4	mmol CH_4 /hr cm^2	% CH_4	mmol CH_4 /hr cm^2
12	10	3.9	4.38	0.0	0.0
	30	3.5	3.93	0.01	0.0
24	10	6.4	7.14	1.0	1.12
	30	3.9	4.38	1.0	1.12
48	10	9.7	5.36	0.7	0.71
	30	12.9	7.14	0.7	0.76
72	10	16.2	8.97	0.1	0.0
	30	13.4	7.41	0.2	0.09

[+] Percent CH_4 in sampling ports which were filled with helium and allowed to equilibrate with porewater CH_4 for a period of 12 or 24 hours after each HRT.

combination of three possible mechanisms, 1) methanogenesis, 2) diffusion of air at the water interface, and 3) O_2 transport through the aquatic plants.

Table 2.3 shows the rate of CH_4 accumulation within the VSBs receiving primary and secondary effluent. There were no consistent differences between rates of CH_4 production at the 10 and 30 cm depths, within and below the root zone (respectively) of Scirpus pungens. There was significant production of CH_4 within VSB receiving primary effluent after a 72 hour retention time even though >95% of the influent CBOD had been removed after 24 hours (Figure 2.3a). Methane production was less in the VSBs receiving secondary effluent. Production reached a maximum after 24 hours, then decreased to almost zero after 72 hours.

Suspended solids

The effluent flow rate and the concentration of suspended solids (SS) in effluent decreased during the 15 minute drain period (Figure 2.4a). The flow rate out of the tank varied due to the falling pressure head (initial water depth was 0.35 m). Flow-weighted concentrations of SS within the VSB and NVSB varied from 20 to 280 mg/L (Figure 2.4b). High SS concentrations were observed after about the 10th batch load. The very high peak (280 mg/L) was not aberrant, but was indicative of a cyclic pattern observed in the batch tanks during four months of previous operation. High concentrations of SS were also observed in the pore

Table 2.4 Mean effluent BOD and CBOD, and CBOD mass removal rates from the vegetated submerged beds (VSB) and floating aquatic macrophyte ponds (FAM) receiving primary wastewater effluent (sample standard deviations are in parentheses).

	Hydraulic loading rate, cm/day				
Treatment	10	10	20	10	20
	BOD	CBOD$^+$			
	mg/L			g/m^2 day	
VSB, gravel					
S. latifolia	18 (10)	7 (4)	5 (1)	17	34
S. pungens	18 (4)	6 (1)	7 (1)	17	34
No plants	20 (7)	7 (1)	na	17	na
VSB, plastic					
S. latifolia	21 (12)	7 (4)	na	17	na
FAM					
H. umbellata	25 (10)	15 (1)	21 (7)	16	31

Influent CBOD and BOD concentration = 176 mg/L.
$^+$CBOD for the systems with a HLR of 10 cm/day was calculated using the following CBOD/BOD conversion ratios; *S. latifolia* = 0.374 ± 0.106 (n = 6), *S. pungens* = 0.333 ± 0.098 (n = 3), *H.umbellata* = 0.624 ± 0.136 (n = 6). Data used for conversion ratios is shown in Appendix A-2.1. CBOD for 20 cm/day represent actual measurements.
na = not available

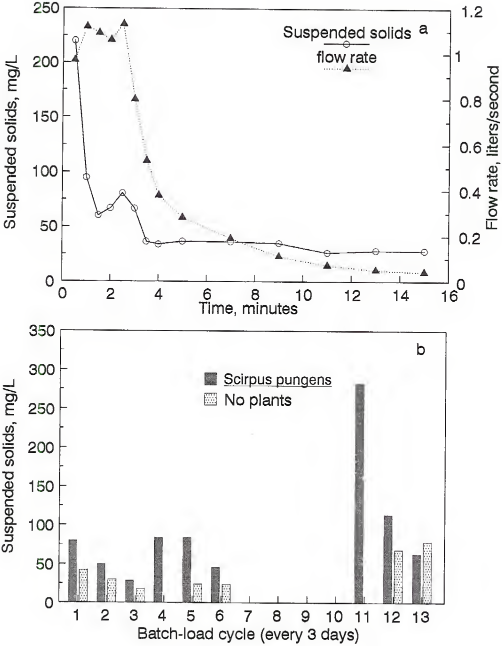


Figure 2.4 Suspended solids (SS) and effluent flow rate from batch-load vegetated and nonvegetated submerged beds during a 15 minute drain cycle. a) SS and effleunt flow rate; b) Flow weighted SS concentrations.

spaces in the continuous-flow VSBs. However, SS were not washed out of the continuous-flow VSB resulting in effluent concentrations less than 30 mg/L.

Nitrogen removal

VSBs receiving primary effluent. Effluent concentrations of NH_4-N in the VSBs (20.7 mg/L) and NVSBs (25.9 mg/L) were higher than the influent NH_4-N (20.3 mg/L). This implies that although 95% of the CBOD was removed within 24 hours in the VSB, O_2 transport through the plants was inadequate for significant oxidation of N during a three day HRT.

VSBs receiving secondary effluent. Effluent concentrations of NH_4-N were lower in the batch-load VSBs than NVSBs (Figure 2.5a). Concentration is a poor indicator of performance of the systems because ET loss from the VSB caused NH_4-N concentrations to be higher than expected. Thus mass removal rates would be a better indicator of system performance. Total N removal increased throughout the 144 hour HRT (Figure 2.5b); however, the mass removal rate of N decreased (Figure 2.5c). If O_2 transport through the plants was a constant process, N removal rate should also have remained constant throughout the 144 hour HRT. The removal rate remained relatively constant in the NVSB (Figure 2.5c). It is possible that low water levels restricted NH_4-N removal by the plants. Although plant

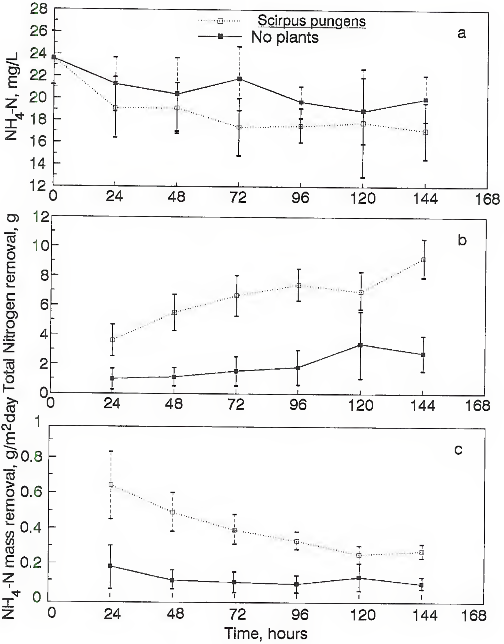


Figure 2.5. Ammonium nitrogen removal from batch-load vegetated and nonvegetated submerged beds receiving secondary wastewater. a) Effluent NH_4-N concentrations; b) Total NH_4-N removal; c) NH_4-N mass removal rate (influent NH_4-N = 25 mg/L).

roots extended the entire depth of the tank, the portion in contact with the water decreased as ET increased.

Experiment II Continuous-Flow VSBs and FAMs

Effect of rooting matrix on BOD_5 removal

Mean effluent BOD_5 concentrations for VSBs with gravel and plastic rooting matrix were equal to or less than 20 mg/L (Table 2.4). Difference in specific surface area between the gravel and plastic rooting media (100 vs 145 m^2/m^3 respectively) had no significant effect on BOD_5 removal at a loading rate of 18 g/m^2 day (Table 2.4).

For the initial two years of operation, the BOD mass loading rate for the VSBs and FAMS was 18 g/m^2 day. When the hydraulic loading rate to VSBs receiving primary sewage effluent was increased to 20 cm/day, the BOD mass load was 35 g/m^2 day. At this higher loading rate, VSBs consistently produced a higher quality effluent than FAMs (Table 2.4). Effluent CBOD concentrations from VSBs were consistently below 15 mg/L, while effluent from FAMs was variable and often above 30 mg/L (Figure 2.6). CBOD mass removal from VSBs were also higher and less variable than CBOD mass removal from FAMs (Table 2.4).

These data indicate that the VSBs consistently produce an effluent low in CBOD at a loading rate of 35 g/m^2 day. However, the front end of the VSBs became clogged and wastewater flowed over the first meter of the VSB surface

Table 2.5 Total nitrogen effluent concentrations and mass removal rates for vegetated submerged beds (with gravel and plastic rooting matrix) and floating aquatic macrophyte ponds (FAMs). Standard deviations are in parentheses.

	Hydraulic loading rate = 10 cm/day						
		Primary			Secondary		
Treatment	n	mg/L		g/m^2 day	mg/L		g/m^2 day
Influent	20	38.1 (5.8)	a	---	32.8 (4.2)	a	---
Gravel							
No plants	10	36.5 (4.3)	a	0.16	29.8 (5.1)	a	0.30
S. pungens	20	34.3 (3.1)	b	0.37	27.9 (7.6)	ab	0.49
S. latifolia	20	31.1 (4.2)	b	0.70	22.3 (6.3)	bc	1.05
Plastic							
S. latifolia	10	32.8 (5.0)	b	0.53	24.6 (6.8)	bc	0.82
FAMs							
H. umbellata	20	33.4 (2.7)	a	0.47	20.5 (7.0)	c	1.23

Note: A General Linear Model t-test (LSD) was used for comparison of treatment means. Different letters in a column are significantly different ($p \leq 0.05$).

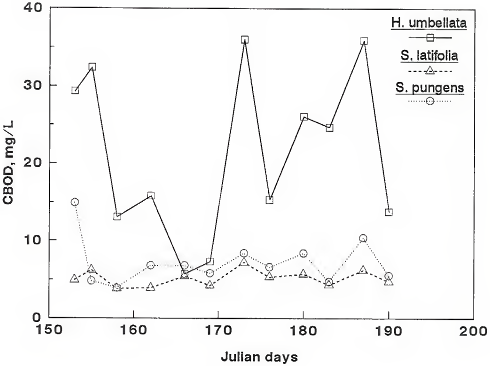


Figure 2.6. Effluent CBOD concentrations in vegetated submerged beds and floating aquatic macrophyte ponds receiving a continuous flow of primary wastewater (hydraulic loading rate = 20 cm/day, mean influent = 210 ± 13 mg/L).

1.5 months after receiving 20 cm/day of wastewater; however, VSBs had been receiving primary sewage effluent for two years prior at a hydraulic loading rate = 10 cm/day. Although clogging did not affect removal of BOD or SS, it does imply that solids management is necessary to maintain flow through the VSB when a high BOD loading rate is applied.

Nitrogen removal

Rooting matrix (gravel or plastic) had no significant effect on total N concentration in VSBs receiving primary or secondary effluent (Table 2.5). Mass removal rate of N was higher for *S. latifolia* planted in gravel compared to plastic when primary or secondary effluent was applied to VSBs. Total N removal rate was higher when influent wastewater was low in CBOD.

Hydraulic characteristics

Hydraulic characteristic curves for VSBs filled with gravel and plastic rooting media (Figure 2.7a and 2.7b, respectively) indicate that neither operate as an ideal CSTR or a plug flow reactor. This is expected in a packed-bed reactor with hydrodynamic dispersion (mass flow and diffusion). The nominal retention time (Eqn [3]) of dye placed in VSBs are shown in Table 2.6. There are no apparent differences between VSBs receiving primary and secondary effluent after six months of receiving wastewater. After one year there were small differences between

Table 2.6 Nominal retention times for Rhodamine WT dye added to vegetated submerged beds (VSB) 6 and 12 months after application of wastewater had begun (hydraulic loading rate was 10 cm/day).

Treatment	Type of effluent	Nominal HRT[+] 6 month	Nominal HRT[+] 12 month
S. latifolia	primary	1.5	1.3
	secondary	1.7	1.4
S. pungens	primary	1.6	na
	secondary	1.7	na
No plants	primary	1.4	na
	secondary	1.5	na
VSB, plastic[♥]			
S. latifolia	primary	1.0	na
	secondary	1.0	na

[+] The nominal retention time of the dye in the VSB is expressed in units of dimensionless time (sampling time/hydraulic retention time).

[♥] Nor-pak plastic media

na = not available

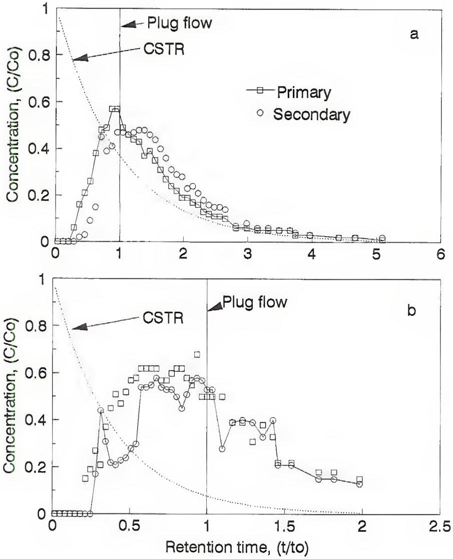


Figure 2.7. Hydraulic characteristic curves for vegetated (Sagittaria latifolia) submergeds beds (VSBs) receiving a continuous flow of primary or secondary wastewater (Hydraulic loading rate = 10 cm/day). Rooting matrix in VSB was a) gravel; b) Norpak plastic media.

hydraulic characteristic curves for VSBs planted with Sagittaria receiving primary or secondary effluent (Table 2.6).

Nominal retention times greater than 1 imply that there was adsorption of the dye onto the gravel and associated solids. Since the nominal retention time of the plastic filled VSB is close to 1 it may be inferred that plastic matrix and roots do not interact with the dye, whereas gravel and solids may have adsorbed dye.

Discussion

CBOD Removal

It is a common assumption that BOD removal in VSBs can be modeled with first-order kinetics (Reed et al. 1988; Wood 1990; USEPA 1990; Cooper 1990; Conley et al 1991). Curve fitting our data to a linearized first-order model gave best fit when HRTs were less than 18 hours. This implies that after 18 hours the assumption of first-order kinetics is not valid, and a different kinetic model is required. Although first-order kinetics were not operating after 18 hours, coefficients were calculated for all data collected for comparison with published data. The rate coefficients observed were higher (some by an order of magnitude) than those previously reported in design manuals for natural systems (Reed et al. 1989; USEPA 1988). The rate coefficients reported by Conley et al. (1991) for VSBs and root zone systems agree closely with those observed in the present study (Figure 2.8). These results suggest that as the HRT increases, the removal rate coefficient decreases. Therefore, VSBs may be operated at shorter HRTs without a loss in CBOD removal efficiency. Shorter HRTs also imply that less land area is required to attain secondary effluent standards for CBOD removal.

Less than 2% of the CBOD was calculated to be oxidized by electron acceptors entering the VSB in the wastewater. Other mechanisms for the removal of CBOD are: (i) methanogenesis, (ii) O_2 transport though the plants, and/or (iii) diffusion of O_2 across the air water interface. An approximation of CBOD removal due to methanogenesis within VSBs receiving primary effluent is possible if, 1) the values in Table 2.3 are assumed to represent the rate at which CH_4 diffuses into the atmosphere at the air water interface, and 2) the pore water is saturated with CH_4 (32.8ml/L). Also, assuming that CBOD = COD, and 1 g COD/393 mL CH_4, it was estimated that 54% of the CBOD removed within 12 hours was due to methanogenesis. These calculations may underestimate the CBOD removal by methanogenesis since CH_4 transport through the plants was not accounted for and may represent 79% of the CH_4 produced in a soil (Cicerone 1981). Fermentation should also significantly reduce the CBOD. The stable methanogenic redox conditions and steady production of CH_4 implies that methanogenesis and fermentation were

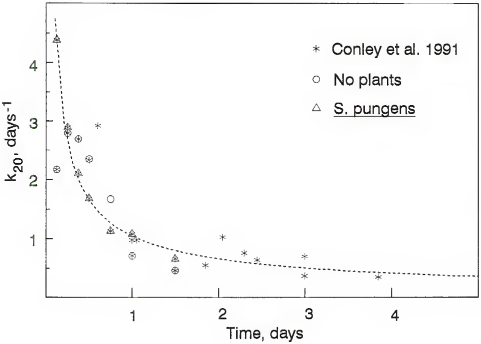


Figure 2.8. Comparison of first-order removal-rate coefficients from systems reviewed by Conley et al. (1990) to those calculated for batch and continuous load experiments.

predominant pathways for oxidation of C. High removal rates of BOD have been reported for anaerobic gravel beds without plants. Young and McCarty (1969) reported a BOD removal rate of 776 g BOD/m^3 day in an anaerobic gravel bed with an HRT of 19 hours. The CBOD removal rate in the VSB after 12 hours was about 400 g CBOD/m^3 day. Comparison to Young and McCarty's research (1969) implies that the VSBs may be capable of higher removal rates, independent of the presence of the plants.

Oxygen Transport

It has been hypothesized that drain and fill cycles would introduce air into the pore spaces of a VSB, thereby enhancing oxidation of organic matter and nitrogen (Seidel 1976; Burgoon et al. 1991; Brix and Schierup 1990). Similar processes have been shown to occur in nature. For example, Howes et al. (1984) has shown that "tidal excursions" in an estuary enhance the oxidation of organic material in an esturarine marsh. During ebb tide, mass flow of air into the pore space provides O_2 which diffuses into the pore water and is consumed in microbial respiration and oxidation of organic matter. It is possible that a similar process may enhance waste treatment in a VSB. Brix and Schierup (1990) hypothesized that mass flow of air into the pore spaces of a rooting matrix will occur more rapidly as the pore space of the soil matrix increases. However, comparison of removal rate coefficients from this research shows that the drain and fill process did not improve waste treatment in the VSB. Analysis of possible mechanisms involved in the storage of O_2 within the gravel during a drain and fill cycle shows why improvement in treatment is unexpected. Since the majority of pore spaces in gravel are large, very little water will remain in the VSB when it is drained (i.e. specific yield is high, Hillel 1982). Therefore, storage of O_2 in pore water retained within micropores, as occurs in the fine textured sediments of the tidal marsh, will occur to a minor extent in a gravel bed. Another possible mechanism for storage of O_2 in the gravel bed would be saturation of microbial biofilms with O_2 while the VSB is drained. An estimate of the amount of O_2 stored in biofilm can be made by assuming a 2 mm thick layer of biofilm covering the gravel. Assuming a water content of 95% in the biofilm, complete saturation (with dissolved O_2) of the biofilm at 25°c, and a specific surface area of 100 m^2/m^3 for the gravel, then less than 2% of the CBOD load would be removed due to entrained O_2. Therefore, both analysis of O_2 transport mechanisms, and results obtained in this study show that batch-load processes do not improve oxidation of organic matter and N.

An estimate of O_2 transport by the plants into the root zone can be calculated if we assume that the difference in C removal between the VSB and NVSB removal was due to O_2 transport through Scirpus pungens. This difference which will be referred to as the apparent O_2 transport rate, is equivalent to 28.6 g CBOD/(m^2 day). This value for CBOD removal is similar to the value of 20 g O_2/m^2 day, that is suggested for removal of BOD in a VSB (Reed et al. 1989).

An enigmatic aspect of the results was that plants significantly affected CBOD and N removal only in the first 12 hours of the treatment period; the effect of plants diminished as the HRT increased. After easily degradable CBOD was removed (95% was oxidized within one day), that O_2 should be available for nitrification. However, there were no changes in NH_4-N or Eh over the 3 day HRT in the VSB or NVSB. The fact that the CBOD continued to decrease over the entire 72 hour HRT in the NVSB, and not in the VSB, also implies that organic compounds may be released by the plants. Other researchers have shown significant differences in C and N removal between VSBs and NVSBs which indicated O_2 transport through the plants (Gersberg et al. 1983; Wolverton et al. 1983). In the present study nitrification was not observed in the VSBs receiving primary effluent with a HRT of 3 days. However, there were significant differences in NH_4-N removal due to nitrification in VSBs receiving secondary effluent.

Nitrogen mass balance in the VSBs receiving secondary effluent shows that less than 10% of the NH_4-N was assimilated into plants during the 144 hour HRT. If the remainder of the N was removed via nitrification and denitrification in the root zone (Reddy et al. 1988), maximum O_2 transport rate would be about 2.4 g O_2/m^2 day. This rate was calculated using the N removal after a 24 hour HRT (Figure 2.5c) and subtracting the N removal for the control. This calculated O_2 transport rate is less than that calculated from the CBOD removal in the VSB. The effect of plants on N removal also decreased as HRTs increased. This method may underestimate O_2 transport because it does not account for oxidation of organic matter. Carbonacaeous BOD was low; however, surrogate methods such as CBOD do not allow accurate accounting because organic material may be released from the roots.

Suspended Solids

A common problem caused by accumulation of solids within VSBs is clogging of pore spaces. Accumulation of solids within VSBs depends on solids loading rate, microbial growth rate, and sedimentation of solids. Operation of VSBs in drain and fill cycles resulted in periodic flushing of solids which accumulated within the VSB. Flushing of solids from the VSB during a drain cycle did not negatively affect subsequent treatment of wastewater. The accumulation of solids in an anaerobic gravel bed has also been reported by Young and McCarty (1969). Young and McCarty (1969) used a synthetic wastewater with low suspended solids; therefore, they suggested solids accumulation was due to biological synthesis. Our results shows that periodic draining of continuous-flow VSBs may remove the accumulated solids and could extend the operating life of the VSB. Further research is needed to evaluate the effects on treatment and disposal of the high solids anaerobic waste effluent.

Accumulation of solids in the continuous flow VSB could not be assessed using hydraulic characteristic curves. A difference was expected between characteristic curves for VSBs receiving primary and secondary effluent due to different loading rates of BOD and SS. The VSBs receiving primary wastewater had BOD and SS loads of 17.6 g BOD/m^2 day and 5 g SS/m^2 day compared to BOD and SS loading rates of 3.3 g BOD/m^2 day and 0.5 g SS/m^2 day for VSBs receiving secondary effluent. Accumulation of solids should result in short circuiting of water over the surface of the VSB and a hydraulic characteristic curve increasingly similar to a complete-mix reactor. However, within one year it was not possible to use nominal retention times to assess accumulation of solids in the VSB. Further research is needed on the effects of SS and BOD loading and their affects on clogging of VSBs.

Effect of Porosity on BOD Removal

These results are similar to studies by Burgoon et al. (1991) which showed that within a range of 100 - 250 m^2/m^3, specific surface area does not have a significant effect on BOD removal within a VSB receiving BOD loading rates less than about 15 g/m^2 day. Due to the large difference in HRT

(80 versus 24 hours for the plastic and gravel respectively) the plastic filled VSB would be expected to remove more BOD than the gravel filled VSBs. These studies showed that BOD removal was not effected by HRT or specific surface area of the rooting media.

Current design manuals suggest that BOD removal is a function of the porosity of the rooting matrix (Reed et al. 1988; USEPA 1990). The following empirical equation for the first-order BOD removal rate coefficient was derived by Reed et al. (1988):

$$k_{20} = k_o \left(37.31\, n^{4.172}\right) \qquad [2\text{-}4]$$

k_{20} = first-order removal rate coefficient at 20°c
k_o = first-order removal rate coefficient in field.
n = porosity

The general applicability of this relationship has not been tested for substrates with a wide range of porosities (such as the plastic and gravel media). The calculated first-order removal rate coefficient (using equation [2-4]) for the plastic rooting matrix is equal to 44.2 d^{-1} compared to a measured removal rate coefficient of 0.58 d^{-1} (calculated from the experimental data with plastic media). This shows that in general, Equation [2-4] cannot be used to calculate removal rate coefficients based on porosity alone. Although this experiment compares two media with extremely different porosities (0.28 vs 0.90 for gravel and plastic respectively), the empirical relationship in Equation [2-4] may also fail if comparisons were made between gravel and a fine textured soil (e.g. clay with a relatively high porosity but low hydraulic conductivity). The low hydraulic conductivity of the fine textured soil will restrict the flow of wastewater through the pores which would result in surface flow of wastewater (Bucksteeg 1987). Consequently BOD removal in a VSB with a fine textured soil would be related to surface area of plant stems not the specific surface area or porosity of the rooting matrix. These results imply that in general, porosity should not be used as a design variable for BOD removal in VSBs.

Conclusions

There were no significant differences between first-order CBOD removal rate coefficients for batch-load and continuous flow VSBs operated with a HRT of one day. The assumption of first-order kinetics was valid only at HRTs less than one day. These data concur with other published literature and show that BOD removal rate coefficients for VSBs may be an order of magnitude larger than recommended in current design manuals. Although BOD removal was significantly higher after 12 hours in the VSB with plants, at longer HRTs plants did not improve the BOD removal.

Periodic draining and filling of the VSB to introduce air into gravel pore spaces did not have any effect on BOD or N removal. Regular draining of the VSB however did cause periodic flushing of solids from the systems.

Oxygen transport rates through plants agreed with rates (20 g/m^2 day) recommended by Reed et al. (1988) for removal of BOD by VSBs. However the apparent effect of plants on BOD removal decreased as the HRT increased. Oxygen transport rates calculated for N were considerably lower than rates calculated for BOD removal in VSBs, and also decreased as the HRT increased. These results imply that O_2 transport is not constant in time, and the rates based on C and N oxidation are significanlty different.

Two rooting matrices with different porosities (0.28 vs 0.90) and specific surface areas (100 vs 140 m^2/m^3) had similar effects on BOD removal. Porosity needs to be used carefully when used as a variable for VSB design.

CHAPTER 3
DESIGN OF CONTINUOUS-FLOW STIRRED TANK REACTORS FOR SIMULATION OF ANAEROBIC WETLAND ENVIRONMENTS

Introduction

During the past decade there has been a growing interest in the use of constructed and natural wetlands for wastewater treatment and water quality improvement (Reddy and Smith 1987; Hammer 1989; Cooper and Findlater 1990). Successful design, construction and use of these systems requires an understanding of the biogeochemical cycles active in the wetlands. Oxygen demand within a wetland soil is generally much higher than diffusion of O_2 through the overlying water. Therefore, molecular O_2 is depleted within the first few centimeters of soil depth. Consequently wetland soils are characteristically anaerobic.

The rates of cycling of carbon (C), nitrogen (N), and sulfur (S) depend on the type of microbial populations present in the wetland. The metabolic needs of these organisms depend on the flux of electron acceptors and donors into the wetland. Types of microbial populations which function in the wetlands are; aerobes, facultative anaerobes, and obligate anaerobes. The bacteria live in a matrix of soil organic and mineral matter permeated by roots of wetland plants. The metabolism and physiology of wetland plants are affected by the extent of anaerobiosis in the soil (Mendelssohn et al. 1981; Mckee et al. 1989).

Wetland plants adapt to soil anaerobiosis by pumping O_2 into the root zone. Oxygen in excess of the root respiratory requirements may diffuse into the rhizosphere and be used by autotrophic and heterotrophic bacteria in the oxidation of C and N (Reddy et al. 1990; Moorhead and Reddy 1988; 1989). Oxygen transport through emergent wetland plants has been verified (Armstrong 1967; Dacey 1981; Grosse and Mevi-Schutz 1987; Armstrong and Armstrong 1990; Armstrong et al. 1990). However, anaerobic root respiration has been shown to be dominant in some wetland plants (Mendelssohn et al. 1981; McKee et al. 1989). These studies have shown that amounts of the fermentative enzyme alcohol dehydrogenase (ADH) in roots are related to the oxidation-reduction (redox) potential of the soil.

Oxidation-Reduction Potential

The most common parameter used to characterize wetland soil anaerobic conditions has been the oxidation-reduction potential (Eh). Redox potential is a measure of the ease with which electrons are transferred from an electron donor to an acceptor. The common donors in soil are organic compounds released during decomposition of plant and animal material. The common electron acceptors in a soil are O_2, NO_3^-, Fe^{+3}, SO_4^{-2}, and CO_2. The amount and types of electron acceptors depends on the organic and mineral content of the soil, and the contents of the hydraulic flows entering the wetland. The driving force for the exchange of electrons between donors and acceptors is the tendency of the free energy of the system to decrease until at equilibrium, when the free energies of the products equals that of the reactants (Ponnamperuma 1972). The driving force can be measured in calories or volts. The change in free energy for the reduction reaction, $Ox + ne^- + mH^+ = Red$, is given by

$$\Delta G = \Delta G_o + \frac{RT}{nF} \ln \frac{(red)}{(Ox)(H^+)^m} \qquad [3\text{-}1]$$

where (red) and (Ox) are the activities of the reduced and oxidized species, and ΔG_o is the free energy when activities are unity (Snoeyink and Jenkins 1980). Converting calories to volts using the relationship $\Delta G = -nEF$, and measuring Eh with a standard hydrogen electrode, Equation [3-1] becomes

$$Eh = E_o - \frac{RT}{nF} \ln \frac{(Red)}{(Ox)} + \frac{mRT}{nF} \ln (H^+) \qquad [3\text{-}2]$$

Equation [3-2] shows that Eh is a function of the activities of the reduced and oxidized species, and the pH of the solution. The standard electrode potentials of the reduction reactions of common electron acceptors in wetlands are given in Table 3.1. The redox couples with the highest free energy are the strongest oxidants. The energy yield available to the bacteria which mediate these

Table 3.1 Oxidation-reduction potentials for redeox couples common in anaerobic wetland soils. Standard cell potentials (Eh_o) are compared to Eh_7 values representative of natural systems (corrected to pH = 7 at 25 ^{O}C). Standard cell potentials are adapted from Zehnder and Stumm (1988); values for natural systems are from (Ponnamperuma 1972).

Reduction half reaction	Eh_o	Eh_7
	Volts	
$O_2 + 4\ H^+ + 4\ e^- \leftrightarrows 2\ H_2O$	1.23	0.33
$NO_3^- + 6/5\ H^+ + 5e^- \leftrightarrows 1/2\ N_2 + 3H_2O$	1.25	0.22
$MnO_2 + 4H^+ + 2e^- \leftrightarrows Mn^{+2} + 2H_2O$	1.22	0.20
$Fe(OH)_3 + e^- \leftrightarrows Fe^{+2} + 3OH^-$	1.06	0.12
$SO_4^= + 9H^+ + 8e^- \rightleftarrows HS^- + 4H_2O$	0.33	-0.15
$CO_2 + 8\ H^+ + 8\ e^- \rightleftarrows CH_4 + 2H_2O$	0.17	na

na = not available

reactions are also greatest from reduction of the strongest oxidants (Stumm 1966).

The reduction of the electron acceptors in wetlands generally occurs in the sequence predicted by thermodynamics and energy yields (Ponnamperuma 1972; Patrick 1981). The observed Eh values listed in Table 3.1 provide a rough guide to the progress of reduction (Ponnamperuma 1972) in a natural soil. Note that potentials measured in natural systems do not agree with expected thermodynamic potentials. Most natural systems consist of a mixture of electron acceptors and donors within a heterogenous mix of microenvironments (Zehnder and Stumm 1988). Therefore, Eh measurements from natural systems represent mixed potentials and may not be interpreted quantitatively (Stumm 1966; Ponnamperuma 1972; Whitfield 1974; Vershinin and Razanov 1983). Despite this fundamental ambiguity in measuring Eh, it is common practice to characterize a wetland soil by measuring the Eh.

Eh has been a useful tool for evaluating spatial patterns and physiological and population responses of wetland plants to anaerobic conditions (Howes et al. 1981; McKee et al. 1989). Simulation of anaerobic wetland conditions in the laboratory have facilitated studies on adaptations of plants to anaerobiosis (Koch et al. 1990; Reddy et al. 1976) and wetland biogeochemistry (Masscheleyn et al. 1991; Patrick et al. 1973; Olila 1992).

In natural systems the availability of electron donors and acceptors, and activity of anaerobic bacteria, determine the Eh. The section that follows introduces an approach to simulating anaerobic wetland environments in the laboratory by establishing anaerobic microbial populations (verified by water chemistry via loss of electron acceptors) as an indicator of anaerobiosis instead of Eh. Accurate simulation of wetland environments requires establishment of anaerobic bacteria and emergent aquatic plants.

Reactor Design for Simulating Wetland Environments

Control of the oxidation-reduction potential

Batch-load reactors have been designed which allow control of Eh and pH in soil suspensions (Patrick 1966; Patrick et al. 1973). These controlled systems have contributed to a basic understanding of redox processes in wetland soils. In these systems, the Eh and pH may be set and controlled at any desired level. The Eh was monitored with platinum and calomel (Hg/HgCl) standard electrodes. Generally, organic carbon (rice straw) was added to the reactors to ensure an excess of electron donors in solution. The inherent direction of thermodynamic equilibrium was towards a methanogenic redox environment. However, in the controlled redox reactor developed by Patrick et al.(1973), when the Eh falls below a desired setpoint (e.g. +200 mV) a meter relay activates an air pump which pumps air into the soil suspension (Delaune et al. 1984). The Eh then increases to the desired set point. Alkaline by-products (from oxidation of organic matter) which accumulate are neutralized by addition of acid to maintain the desired pH level. Pseudo-steady state conditions are maintained within the reactors for about 15 days. Under these conditions several redox couples in different soils have been studied (Connell and Patrick 1968; Moraghan and Patrick 1974; Cleemput et al. 1975). Current research with these reactors has addressed arsenic and selenium chemistry as affected by sediment Eh and pH (Masscheleyn et al. 1991; 1990). A modified version of the reactor (Reddy et al. 1976) allows for introduction of plants for studying effects of Eh on plant growth, root respiration, and nutrient cycling (Delaune et al. 1984). They have also been used to study phosphorus cycling in a eutrophic lake (Olila 1992).

Although this approach for simulating redox environments has been useful in studying the basic redox processes in wetland soils (Patrick 1981), the approach used to control Eh is a poor simulation of natural wetlands. The major drawback is that Eh is controlled using O_2 in the simulated anaerobic environment. This does not occur in reduced environments. Pulsing O_2 into a soil system affects decomposition rates of organic matter and rapidly oxidizes reduced compounds (e.g. NH_4-N, Fe^{+3}, Mn^{+2} etc.). The bacterial ecology may also be altered since aerobic bacteria may become established. Studying oxidation of organic matter becomes obfuscated since facultative anaerobes prefer O_2, and O_2 is toxic to obligate anaerobes (Widdel 1988).

This chapter introduces a reactor designed to control Eh and pH in an anaerobic environment. The goal was to create an environment which was representative of an anaerobic wetland. The Eh was poised by the oxidants and reductants in solutions which are representative of redox processes as they occur in wetlands. The Eh of the systems was defined by the chemical species and pH of the solution according to the Nernst equation ([3-3]).

Hydraulic considerations in reactor design

Two ideal hydraulic conditions which are used to model reactors are plug flow and complete mix hydraulics (Metcalf and Eddy 1979). The hydraulic conditions affect the metabolic status of the bacteria and chemical equilibrium in the reactors. The bacteria and chemistry of the reactors can be in a steady state (conditions do not change in time) or dynamic state (changing with time). If a solution continuously flows into the reactor, whether it is plug flow or complete-mix, the bacteria and consequently the chemistry will come to an equilibrium which will be referred to as steady state. The reactor designed by Patrick (1966) to control Eh and pH in soil suspensions was a complete mix batch reactor. Bacterial properties and chemistry in batch reactors constantly change as the resources are depleted and by products accumulate, they are therefore, dynamic (change in time). Plug flow may be simulated in columns packed with porous media. Complete-mix hydraulics are simulated in reactors which are continuously stirred. An advantage of complete mix conditions in a continuous-flow stirred tank reactor (CSTR) is that steady state conditions are established. The reactor design being presented consists of a column packed with porous media, an arrangement which will generally result in hydraulic conditions similar to plug flow. However, complete-mix conditions can be created in a packed column by recycling the reactor effluent at a recycle rate (Q_R) which is much faster than the influent flow rate (Q_{in}) (Characklis and Marshall 1990). This technique has been used in columns used to simulate ground water environments in packed columns (Rittman et al. 1986; Wrenn 1992). A continuous-flow of electron donors and acceptors through the completely-mixed reactor theoretically creates conditions of steady state chemistry and bacterial metabolism.

The objectives of this study were to: (i) develop a continuous-flow stirred tank reactor for growing emergent aquatic plants (Experiment 1); (ii) establish steady state biofilms of nitrate- and sulfate-reducing bacterial communities with controlled Eh and pH within the reactors (Experiment 2a and b); and (iii) establish steady state levels of oxidizable C and N which may be used as indicators of O_2 transport through the plants.

Materials and Methods

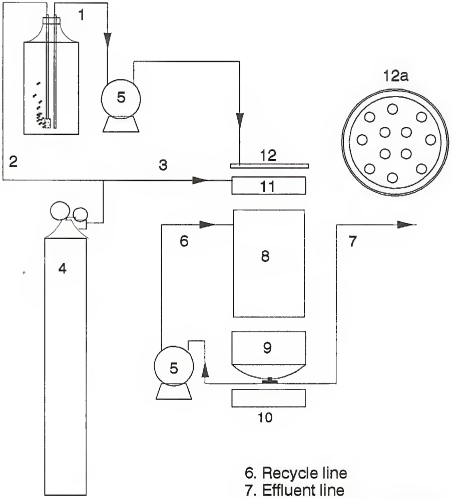
Experiment I. Design and Hydraulic Characteristics of Continuous-flow Stirred Tank Reactors with Anaerobic Biofilms

The objective of this experiment was to (i) develop design of the CSTR; and (ii) determine if the designed reactor functions as a CSTR. The hypothesis was that by varying the recycle ratio, complete-mix conditions could be created in a reactor packed with porous media.

Reactor construction and operation

An expanded view of the reactor, and supporting equipment are shown in Figure 3.1. A total of 6 reactors were built, two had 4.6 L pore volumes, the other four had 2.2 L pore volumes. Physical and operational characteristics of the two reactor types are shown in Table 3.2.

The reactor was built with 15.2 cm (6 in.) I.D. schedule 40 PVC pipe. A 15.2 cm PVC end cap was glued to one end of the pipe. A 2.54 cm (1 in.) section of 15.2 cm I.D. pipe was glued to the end cap to create a stand for the reactor. The lid for the reactor was made by gluing a 0.64 cm (1/4 in.) thick Plexiglas disc on top of one half of a 15.2 cm I.D. PVC pipe couple. Silicone grease was applied to the inside flange of the couple to create an airtight seal when the lid was placed over the pipe. Holes were cut into the Plexiglas disc (2 cm I.D.) for placement of



1. Nutrient solution delivery
2. N_2 gas line
3. N_2 gas to purge headspace
4. N_2 gas, 99.9 % pure
5. Cole-Parmer peristaltic pump
6. Recycle line
7. Effluent line
8. 15.2 cm (6 in) I.D. PVC pipe
9. 15.2 (6 in) I.D. PVC end cap
10. 15.2 cm (6 in) I.D. PVC pipe
11. 1/2 of a 10.2 cm PVC couple joint
12. 3.2 mm (1/8 in) plexiglas lid

12 a. Holes in plexiglas lid

Figure 3.1. Exploded view of physical components of a continuous-flow stirred tank reactor used to simulate nitrate- and sulfate-reducing wetland environments.

Table 3.2 Physical characteristics of continuous-flow stirred tank reactors used to simulate nitrate- and sulfate-reducing wetland environments. Two different sized reactors were designed, each had the same basic configuration as in Figure 3.1. In the text, reactors will be referred to by void volume (i.e. 2.0 and 4.6 L).

Dimension	Symbol (units)	Design 1	Design 2
Reactor length	L, (cm)	30	12
Diameter	d, (cm)	15.2	15.2
Cross-sectional area	A, (cm^2)	182	182
Total volume	V_T, (cm^3)	5550	2240
Void volume, measured	V_v % (cm^3)	84 4600	90 2000
Void volume,	V_v, (%)	90	90
specific surface area of Tri-Pak[1]	A_s, (cm^2/cm^3)	2.8	2.8
Specific surface area of reactors[2]	cm^2/cm^3	4.6	4.4
Flow rate	Q_{in}, (L/day)	1.63	1.15
Retention time	HRT, (day)	2.8	1.7
Recycle flow rate	Q_R (L/day)	315	315
Recycle ratio	Q_R/Q_{in}	193	274

[1] Void volume and specific surface area of plastic as reported by manufacturer (Jaeger Tri-Paks, Inc., Fountain Valley, CA.

[2] Calculated using (V_v manufacturer/V_v measured)*A_s

influent nutrient solution lines, gas lines, pH and Eh electrodes, and plants (Figure 3.1). Stoppers (size no. 3) were placed in the holes of the lid for the reactor which did not have plants.

Masterflex variable speed peristaltic pumps (Model 7567-70, Cole-Parmer, Chicago IL.) were used to continuously feed the nutrient solution to the reactors and to recycle the solution in the reactor. The recycle flow was returned to the top of the reactor 1.5 cm below the water level. Water level in the reactor was maintained about 2 cm below the lid of the reactor by adjusting the height of the effluent tube (Figure 3.1). The influent nutrient solution dripped into the reactor at the same point that the recycle flow entered the reactor to facilitate mixing. Norprene flexible tubing (Cole-Parmer size 18, I.D.= 7.9 mm) was used for the recycle lines. The headspace was continuously purged with 99.99% N_2 at a flow rate of 80 - 100 ml/min to minimize flow of O_2 into the reactor headspace and remove gaseous H_2S.

Surface for attachment of biofilms. Bacterial cultures can be established either as a suspension, or with bacteria attached to a fixed surface (biofilms). This reactor was designed as a CSTR with anaerobic biofilms.

The CSTRs were filled with Tri-pak plastic packing media (Jaeger Inc., Spring, Texas). The Tri-pak plastic balls are 2.5 cm in diameter with a specific surface area of

280 m^2/m^3, and a measured pore volume of 50 % (Figure 3.2). The plastic media was chosen instead of a soil for the following reasons: (i) the low surface area relative to a soil (280 m^2/m^3 for plastic vs 6000 m^2/m^3 for a sandy soil with a mean diameter of 0.1 cm) limits the size of the bacterial population in the reactor[3], (ii) high porosity facilitates high recycle rates, and (iii) plastic is an inert media and does not supply C to the bacteria. Therefore, C balances and steady state chemistry are more easily achieved.

The Tri-pak are slightly buoyant, therefore, vexar plastic mesh was used to submerge the tri-pak 2-3 cm below the water level in the reactor (Figure 3.3).

Reactor hydraulics. The hydraulic flow patterns were tested by adding a conservative tracer (Rhodamine WT) to the two 4.6 L reactors. Theoretically, a conservative tracer is not decomposed or adsorbed within the reactor. This was a good assumption because sulfate-reducing bacteria isolated from wastewater metabolize only small volatile fatty acids (Widdell 1988), and Rhodamine WT has low adsorptive properties (Smart and Laidlaw 1977). Effluent concentrations of tracer were compared to the curve for an

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[3] Monod model for microbial growth shows that the rate of substrate utilization is directly proportional to microbial biomass (Metcalf and Eddy 1979).

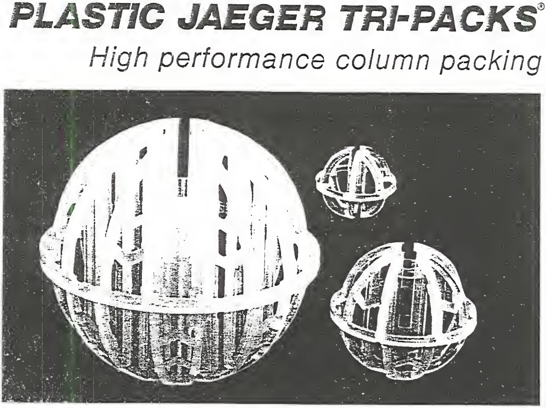
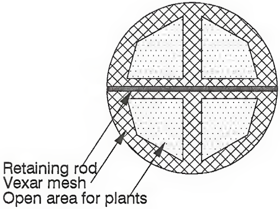
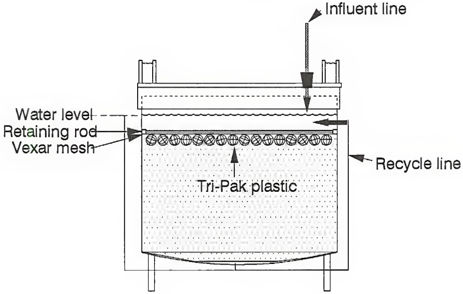


Figure 3.2. Tri-pak plastic media used as a surface for attachment of nitrate- and sulfate-reducing bacteria, and rooting matrix for Scirpus validus grown in continuous-flow stirred tank reactors.



b). Plan view



a). Cross-section

Figure 3.3. Detail of interior of continuous-flow stirred tank reactor filled with buoyant Tri-pak plastic balls. Retaining rod and vexar mesh were used to hold plastic 2-3 cm below water level. a) Cross section of reactor; b) Plan view of vexar mesh and retaining rod.

ideal CSTR (equation [3], Metcalf and Eddy 1979) using methods from Middlebrooks et al. (1982):

$$C_e = C_o * e^{-t/to} \qquad [3-3]$$

where C_e = effluent concentration
C_o = influent concentration
t = time of sampling
t_o = hydraulic retention time.

The influent flow rate was set at 3.5 mL/min while the recycle rate was adjusted on the variable speed Cole-Parmer peristaltic pump. Recycle ratios tested were 62, 70, 77, and 88. After a recycle ratio was set, 300 μL of 2.4 x 10^3 mg/L Rhodamine WT fluorescent dye was injected into the reactor at the same point that the influent entered the reactor. Effluent samples were collected (as it dripped out of the effluent line) every 15 minutes for the first 3 hours, then on the hour for the next nine hours. After the initial 12 hours samples were collected intermittently for up to 5 days (5 HRTs). Fluorescence of the effluent samples was measured using a Turner Design Fluorometer.

A homogeneous chemical environment is also an indicator of complete mix conditions in a CSTR. Homogeneity was evaluated by testing for stratification within the reactor. Nonconservative reactants such as electron donors (acetate) and acceptors (SO_4-S) in the reactors were sampled at 5 cm depth increments in the 4.6 and 2.0 L reactors. Oxidation-reduction potential was also evaluated with depth. There were no plants in the reactors during the studies on hydraulic characterization.

EXPERIMENT II. Steady State Culture of Nitrate- and Sulfate-reducing Biofilms with Scirpus validus

The experimental objective was establishment of steady state nitrate- and sulfate- reducing biofilms within the CSTRs designed in Experiment 1. The hypothesis was that introduction of emergent aquatic plants into the steady state anaerobic microcosms will cause detectable changes in concentrations of acetate, SO_4-S, NO_3-N, Eh, and/or pH.

Experimental setup

One 4.6 L, and two 2.0 L reactors were used for growing the sulfate-reducing biofilms, a similar set of reactors was used for culturing bacteria. The hydraulic studies conducted on the 4.6 L reactors showed that a minimal recycle ratio of 88 was necessary for complete mix conditions. The recycle ratios for reactors used in these experiments were greater than 190 (Table 3.2). This was accomplished by using influent flow rates which were lower than those used in experiment 1 (1.1 or 0.8 vs 3.2 mL/min).

Data presented are for periods when reactors were stable and approached steady state conditions for at least 30 days (Table 3.3). During PHASEs I, II, and III, two 2.0 L reactors were used to culture SRB. During PHASEs II and III, two 2.0 L reactors were used to culture NRB. During PHASE IV one 4.6 L reactor was used to culture SRB and one to culture NRB. Reactors in PHASE I were left without plants to establish baseline operations. Twenty-seven days after initiation of PHASE II, Scirpus validus was planted in one NRB and one SRB reactor. The plants were in the reactors for all of PHASE III. After 37 days plants were removed from the reactors in PHASE IV. Relative loading rates of acetate, NO_3-N and SO_4-S varied from 1 to 1/3 during PHASEs I through IV. The effects of different loading rates of C, N, and S were varied (actual mass loading rates are shown in Table 3.4) and analyzed for affects on steady state Eh, pH, and effluent concentrations of electron donors and acceptors.

Bacterial inoculation

The reactors were initially filled with settled secondary wastewater (nitrate-reducing biofilm reactor [NRB]), or settled primary wastewater (sulfate-reducing biofilm reactors, [SRB]) obtained from the University of Florida Wastewater Treatment Plant. Over a period of three weeks the feed solution was changed from 100% settled wastewater to 100% nutrient solution.

Nutrient solution

Nutrient solutions were prepared in opaque brown 8 L carboys. A #9 rubber stopper was place in the mouth of the carboy. Three 2 mm holes were bored in the stopper, holes were for a N_2 gas line, an escape hole for N_2 gas, and a nutrient solution feed line (Figure 3-1). Nutrient solution

Table 3.3 Treatments and experimental conditions during an experiment with anaerobic nitrate- and sulfate-reducing (NRB and SRB respectively) continuous-flow stirred tank reactors during Experiment II.

PHASE	Dates	Experimental conditions				
				Relative loading rates		
		Anaerobes[+]	Treatment	Acetate[♥]	NO_3-N [♦]	SO_4-S [♣]
I	8/29 to 12/7/91	SRB only	no plants	1	na	1
II	3/15 to 7/1/92	NRB and SRB	no plants/ plants[♠]	1	1	1/2
III	7/7 to 9/7/92	NRB and SRB	no plants/ plants	1/3	1/3	1/5
IV	3/6 to 5/20/92	NRB and SRB	no plants/ plants[⊚]	2/3	2/3	1/2

[+] During each phase (except PHASE I) two reactors were used for each anaerobe type).
[♥] Actual loading rates of acetate are presented in Table 3.4.
[♦] Pertains only to nitrate-reducing reactors
[♣] Pertains only to the sulfate-reducing reactors
[♠] Plants were put in reactors 4/12/92
[⊚] Plants were removed from reactors 4/12/92

Table 3.4 Hydraulic retention times (HRT) and mass loading rates (mg/L*hr) of carbon (C), sulfur as SO_4-S (S), and nitrogen as NO_3-N to the continuous-flow stirred tank reactors during four phases of operation.

	PHASE I	PHASE II	PHASE III	PHASE IV
Q_{in} (mL/hr)	48	48	48	66
volume (L)	2.0	2.0	2.0	4.6
HRT (hrs)	41.7	41.7	41.7	69.7
SO_4-S REDUCING	Mass loading, mg/L hr			
Acetate	12.2	11.5	3.7	8.5
C	5.0	4.7	1.5	3.5
S	4.1	2.3	0.8	2.3
NO_3-N REDUCING				
Acetate	na	12.2	3.7	8.5
C	na	5.0	1.5	3.5
NO_3-N	na	3.1	1.0	1.9
S	na	0.2	0.07	0.11

Mass load = $C_{in} * Q_{in} / V = C_{in} / HRT$

was pumped continuously at a rate of 1.1 and 1.8 mL/min into the 4.6 liter reactors and 0.8 mL/min into the 2.0 liter reactors. Norprene flexible tubing[4] (Cole-Parmer size #13, I.D. = 0.8 mm) was used to deliver nutrient solution to each reactor.

Anaerobic conditions were maintained by continuously purging the nutrient solution with 99.99% pure N_2 gas (80 - 90 ml/min). A wooden fine bubble diffuser was used to enhance stripping of O_2 from the nutrient solution. Copper tubing was used for all gas lines from the compressed gas cylinder to minimize diffusion of O_2 into the N_2 gas.

Maximum specific growth rate of the bacteria was not desired, therefore the nutrient solution was formulated to supply the minimum nutritional requirements for microbial growth (Widdel and Pfennig 1989). Minerals were also added to provide the minimal requirements of plants. Nutritional guidelines for wetlands plants are not established, therefore nutritional requirements of vegetable crops presented by Resh (1981) were used as a guide for preparation of the nutrient solution. The pH of the nutrient solution in the 8 L carboys was adjusted with concentrated hydrochloric acid (Table 3.5).

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[4] oxygen permeability of the norprene tubing is 5.49 mL/mm thickness/cm_2 day atm (90 ml/mil thickness/100 in^2*day*atm) as reported by Cole-Parmer Inc..

Table 3.5 Nutrient solution pH for nitrate- and sulfate-reducing reactors during four phases of operation. Volumes of concentrated hydrochloric acid added to each 8 liter carboy of nutrient solution are shown.

	Sulfate-reducing CSTRs[+]			Nitrate-reducing CSTRs		
PHASE[♥]	NPL[♦]	PL	PL	NPL	PL	PL
PHASE I	2.92 (.16)	2.78 (.23)	na	2.96 (.72)	2.93 (.68)	na
mL HCL	14	14	na	14.5	14.5	na
PHASE II	3.01 (.01)	3.01 (.01)	na	2.15 (.12)	2.36 (.71)	na
mL HCL	8.8	8.8	na	13.0	13.0	na
PHASE III	3.16 (.11)	3.37 (.30)	na	2.74 (.08)	2.71 (.02)	na
mL HCL	3.9	3.5	na	4.6	4.6	na
PHASE IV	na	na	3.08 (.29)	na	na	2.33 (.30)
mL HCL	na	na	5	na	na	7

na = not applicable

[+] Mean and standard deviation (in parentheses)

[♥] 2.2 L reactors were used during PHASES I, II, and III; 4.9 L reactor was used during PHASE IV

[♦] NPL = no plants, PL = *Scirpus validus*.

Nutrient solution for sulfate-reducing cultures. Sulfate was added as the only electron acceptor (Table 3.6). Yeast extract was added (1 % of carbonaceous chemical O_2 demand) to supply required growth factors (Widdel and Pfennig 1984). Use of acetate as an electron donor favored selection of acetate-oxidizing SRB. Concentrations of macro- and micronutrients used in the basic nutrient solutions are shown in Table 3.6. The influent concentration of acetate, SO_4-S, NO_3-N, and Fe^{+2} was varied during the phases of operation (Tables 3.8, 3.10). Composition of stock solutions and volumes added to the 8 L carboys are shown in Table A-3.1.

Control of sulfides. Microbial reduction of one mole of SO_4 results in production of one mole of sulfide. Establishing plants in the sulfate-reducing reactors requires sulfide concentrations at levels nontoxic to the emergent aquatic plants. Methods to control sulfides in solution were based on sulfide chemistry. Speciation of sulfides in solution are dependent on pH as shown in Equation [3-4]:

$$H_2S_{(g)} \leftrightarrows H_2S_{(l)} \leftrightarrows HS^- + H^+ \leftrightarrows S^= + H^+ \qquad [3-4]$$

As pH decreases gaseous hydrogen sulfide is formed. In current reactor conditions (conductivity = 2200 μmhos/cm and T = 25°c), the percent of total sulfur as $H_2S_{(l)}$ at pH = 6, 6.5 and 7.0, are 90, 72 and 50 % respectively (APHA 1989). Volatilization of the $H_2S_{(l)}$ is a function of the partial

Table 3.6 Basic composition of nutrient solution for nitrate- and sulfate-reducing continuous-flow stirred tank reactors. Note in Tables 3.8, and 3.10 that influent concentrations of acetate, sulfate, and nitrate change during different phases of operation.

Salt	Nutrient	SO_4-S reducing CSTR	NO_3-N reducing CSTR
		---------- mg/L ----------	
$NaC_2H_4O_2$	Na	272	272
	$C_2H_4O_2$	694	694
K_2SO_4	K	142	0
	S-SO_4	58	0
NH_4SO_4	N-NH_4	1.5	0
	S-SO_4	3.5	0
$FeSO_47H_2O$	Fe^{+2}	107	2.2
	S-SO_4	62	1.2
K_2HPO_4	K	71	71
	P	28	28
$CaCl_22H_2O$	Ca	57	57
	Cl	100	100
$MgCl_26H_2O$	Mg	19	19
	Cl	55	55
$MnCl_24H_2O$	Mn	1.2	1.2
	Cl	1.5	1.5
$ZnCl_2$	Zn	1.2	1.2
	Cl	0.1	0.1
KI	K	0.5	0.5
	I	1.6	1.6
NH_4Cl	N-NH_4	12	0
	Cl	4.2	0
KNO_3	K	0	485
	N-NO_3	0	173
NH_4NO_3	N-NH_4	0	9
	N-NO_3	0	9
Yeast extract	--	3	0

Preparation of stock solutions added to the 8L carboys to attain the above concentrations are presented in Table A-3.1

pressure of $H_2S_{(g)}$ in the headspace above the reactor. Therefore the concentration of sulfides in solution can be controlled by adjustment of pH, and continuous purging of the head space in the CSTR which maintains low partial pressure of $H_2S_{(g)}$, which enhances volatilization of $H_2S_{(l)}$. Ferrous iron was also added to the nutrient solution for precipitation of sulfides (Snoeyink and Jenkins 1980) (Figure 3.6). The amount of Fe^{+2} added was in excess of the requirements of plants and bacteria to insure effective precipitation of sulfide.

Nutrient solution for nitrate-reducing bacteria. Nitrate was added as the sole electron acceptor, with acetate as the only available electron donor. Ferrous Fe and SO_4-S were added at levels required as micronutrients for plants (Resh 1981) and bacteria (Austin 1988). Other nutrient additions were the same as those added to the sulfate-reducing reactors, except that no yeast extract was added (Table 3.6) because NRB do not need the growth factors (Widdel and Pfennig 1984).

Plant growth

The longstem bulrush, *Scirpus validus*, was propagated from seed, planted in gravel and grown in a shaded greenhouse. Plants were fed a mixture of nutrient solutions used for growing the nitrate- and SRB. Five segments of roots and rhizome (about 3 cm each) with 2 to 5 tillers/rhizome were removed from the gravel. The roots were

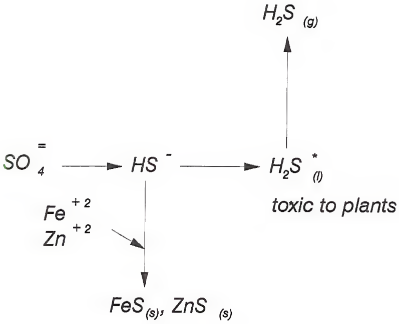


Figure 3.4. Sulfide levels in the sulfate-reducing reactors were controlled by maintaining low partial pressure of H_2S, precipitation with Fe^{+2}, and adjustment of pH.

rinsed thoroughly with water, the tillers were wiped with a Benomyl (fungicide) solution, and then plants were placed in the reactors. The roots were placed 4-5 cm deep into the Tri-pak plastic media, so that the crown of the roots was below the top of the media. The tillers ranged from 2 to 30 cm in height. Six 1.2 meter (four feet) fluorescent bulbs provided approximately 170 μEinsteins/cm^2*sec of light at the top of the plants, and 65 μEinsteins/cm^2*sec at the base of the plants. Light was measured with a Licor Model 220 Quantum sensor. Plants were grown in the reactors for several months (Table 3.3). Rope caulk wrapped in parafilm was placed around the base of the plants to minimize leakage of air around the plants and into the reactor (Moorhead and Reddy 1988).

Water sampling and analysis

Water samples were taken every 2-3 days from a depth of about 2 cm below the water surface. Influent samples were collected from the nutrient solution feed lines as they entered the reactor. All samples were filtered through 0.45 μm filters and stored at 4°C in 7 mL scintillation vials until analysis.

Acetate, NO_3-N, and SO_4-S. Acetate, NO_3-N, and SO_4-S were analyzed using a Dionex 4500i Ion Chromatograph with conductance detector, and anion micro-membrane suppressor (AMMS-1). Separation from other anions was accomplished using an Ionpac AS4A analytical column and an Ionpac AG4A guard column. A 1.8 mM Na_2CO_3/1.7m M $NaHCO_3$ eluant solution was used for separation of NO_3-N and SO_4-S; a 5 mM tetraborate eluant solution was used for the separation of acetate.

Oxidation of sulfides. Oxygen may diffuse through the plastic scintillation vials, therefore oxidation of sulfides to sulfate may interfere with measurement of SO_4-S. Sulfides may be oxidized via chemical and/or biological pathways. Chemical oxidation of $S^=$ to SO_4-S was unlikely at a pH = 6-7, the pH range in the samples (Almgren and Hagstrom 1974; Chen et al. 1972; Cline and Richards 1969). Low concentrations of $S^=$, filtration of samples through 0.45 micron filters, storage in the dark, and immediate analysis for SO_4-S decreased potential interference of $S^=$ oxidation in the samples.

Iron: total and Fe^{+2}. Water samples were filtered through 0.45 μm filters into a 50 mL erlenmeyer flask fitted with a rubber septum. The flask was continuously purged with N_2 gas (250 ml/min) to maintain anaerobic conditions. Samples were analyzed for total Fe and Fe^{+2} using the Phenanthroline Method (#3500-Fe D, APHA 1989). A Shimadzu Spectrophotometer Model UV-160 was used to determine transmittance through the samples. Standard Fe^{+2} solutions were prepared ranging in concentration from 0.2 to 1.4 mg/L Fe^{+2}. Ferric Fe concentrations were obtained from the difference between Fe^{+2} and total Fe concentrations.

Total and soluble sulfides. The iodometric titration technique (method #4500-S^2-E , APHA 1989) was used to determine sulfides. Samples for the analysis of sulfides were obtained using the same methods described for Fe analysis. Samples were removed from the 50 mL erlenmeyer flask and immediately injected into a 250 mL erlenmeyer flask containing an acidified iodine solution and then diluted to 150 mL and titrated with 0.025 M sodium thiosulfate. Total soluble sulfides were obtained by filtering (0.45 μm filter) the samples as they were injected into the 50 ml flask. A nonfiltered sample was analyzed to yield total sulfides in solution.

Physico-chemical parameters. A Corning deep cell pH electrode and Orion pH meter were used to measure the pH. After several minutes of immersion at 2 cm depth, a pH reading was recorded. The electrode was stored in deionized water and calibrated regularly with buffer solutions ranging from pH = 4 and 7 using an Orion pH meter.

The redox electrode and reference cell were prepared in the laboratory. Redox electrode consisted of a one cm piece of platinum wire (#18 gauge) embedded in a glass tube. Mercury was placed inside the glass tube as an electrical contact with #16 gauge copper wire. The reference electrode was a mercury/mercurous chloride calomel cell. Measurements were taken by placing the tip of the platinum wire about 2 cm below the water surface in the reactor. The electrode was calibrated with quinhydrone in buffer solutions of pH = 4 and 7; the calibration redox values were 41 and 216 mV, respectively. Platinum electrodes are susceptible to sulfide poisoning, therefore the electrodes were left in the reactor for just a few minutes until a stable reading was obtained. After use they were rinsed with distilled water, dried, and polished with emory paper to remove sulfide or chloride precipitates that may have formed on the electrode (Whitfield 1974).

Conductivity of the samples was measured with a YSI Conductivity meter.

Temperature around the reactors was maintained at 25°C ± 1°C.

Total and microbial solids

Three methods were used to assess microbial biomass. The first involved subsampling the Tri-pak plastic balls used as a surface for biofilms. The attached solids, from 9 to 12 balls per reactor, were scrubbed off with a test tube brush, then oven dried at 100°C (total solids) and ashed at 500°C (total volatile solids). Total volatile solids was assumed to represent the portion of microbial biomass in the total solids (method # 2540-E, APHA 1989). Mean biomass per tri-pak plastic ball was calculated, then multiplied by the number of balls in the reactor to estimate the total biomass in the reactor.

The second method involved washing the solids out of the reactor. The recycle pumps were turned off and the reactor contents were then vigorously stirred and rinsed several times. The solids were settled in Imhoff cones then separated and dried at 80°C for 24 hours. This method removed loosely attached biofilm and left a small amount of biofilm for maintenance of the cultures. Subsamples of the dried solids were ashed, difference between dried and ashed weights were used to calculate volatile solids.

A third method involved analysis of oven dried solids on a Carla Erba NA-15-C,N, and S analyzer (Haake-Buchler Instruments, Saddlebrook, N.J.). Microbial biomass was calculated by assuming a molecular composition of $C_5H_{12}N_2O_3$ for the bacterial cells (McCarty 1975). All C and N measured was assumed to be from microbial biomass.

Statistical methods

Comparison of water quality parameters were made using the Statistical Analysis Systems version 6.04 (SAS 1987). A Generalized Linear Model and/or Analysis of Variance was used to compare the differences between treatments. The null hypothesis was that Observation 1 (at t_i in control) - Observation 2 (at t_i in reactor with plants) = 0, i.e. there were no differences between the treatments.

Results

Experiment I. Design and Hydraulic Characterization of CSTRs

Hydraulics

Four recycle ratios (Q_R/Q_{in}) were evaluated for the 4.9 L reactors. Complete mix conditions were simulated at the highest recycle ratio (Figure 3.5). The optimum recycle ratio was 88, and turn over time was 15.8 minutes. Therefore, within one HRT the reactor contents were turned over about 91 times. It was assumed that a recycle ratio greater than 88 (optimum for 4.9 L reactor) would ensure complete mix conditions in the 2.2 L reactors. The operating recycle ratio used for the 2.2 L reactors ranged from 190 to 280, with a turnover time of approximately 10 minutes. The large recycle rate was possible due to smaller reactor volume and subsequent lower influent flow rate.

Chemical homogeneity

The SO_4-S concentration decreased with depth in both 4.9 and 2.2 L reactors. The maximum difference between two depths was 1.0 mg/L in the 2.2 L reactors and 0.5 mg/L in the 4.9 L reactors (Figure 3.6a). These differences were small compared to the influent concentration of 178 ± 7 mg/L.

Acetate concentrations showed no consistent trend with depth. Although some stratification was observed on one sampling day, the variation of 6.2 mg/L was small compared

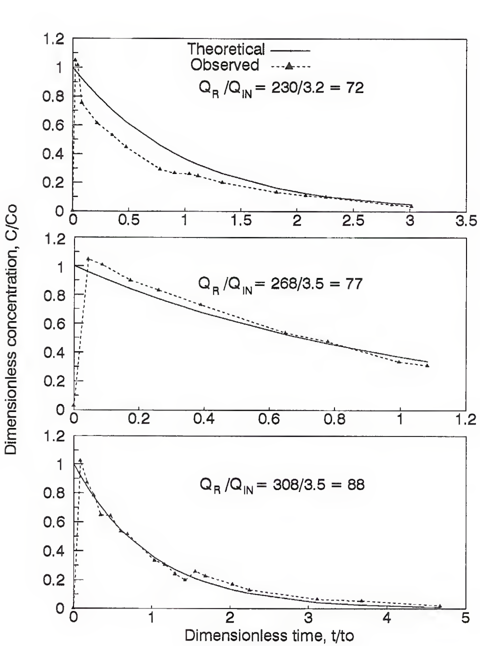


Figure 3.5. Hydraulic characteristic curves for the 4.9 L continuous-flow stirred tank reactors (CSTR) operated at three different recycle ratios. A conservative tracer, Rhodamine WT, was added to the CSTRs. Experimental data are compared to a theoretical curve for an ideal CSTR.

to the influent concentration of 602 ± 9 mg/L acetate (Figure 3.6b). The variation with depth was <1% of the influent concentrations of SO_4-S and acetate (Figure 3-6a and b).

A depth profile of Eh was measured in two of the 2.2 L reactors. There was a difference of about 100 mV in both reactors between the 3 and 12 cm depths (Table 3.7).

Experiment II. Steady State Nitrate- and Sulfate-reducing Cultures

Experiment IIa. Steady state nitrate-reducing cultures

Acetate and N loading rates. The stoichiometric C/N ratio for complete oxidation of acetate with nitrate was 1.07. The C/N ratios used throughout PHASEs II, III, and IV, ranged from 1.37 to 1.56 (Table 3.8). The acetate loading rate varied from 3.9 to 14.0 mg/L*hr, and the NO_3-N loading rate ranged from 0.73 to 4.0 mg/L*hr (Table 3.4). At all loading rates greater than 99 % of the acetate was removed. The average concentration in all reactors was less than 5 mg/L acetate and 1 mg/L NO_3-N (Table 3.8).

Fe and SO_4-S. The influent concentrations of SO_4-S ranged from 3 to 12.5 mg/L (Table 3.8) during PHASEs II, III and IV. The total influent Fe ranged from 2.5 to 3.1 mg/L. Throughout all phases, concentrations of SO_4-S decreased by at least 86 % in reactors with and without plants (Table 3.8). There was no apparent effect of plants

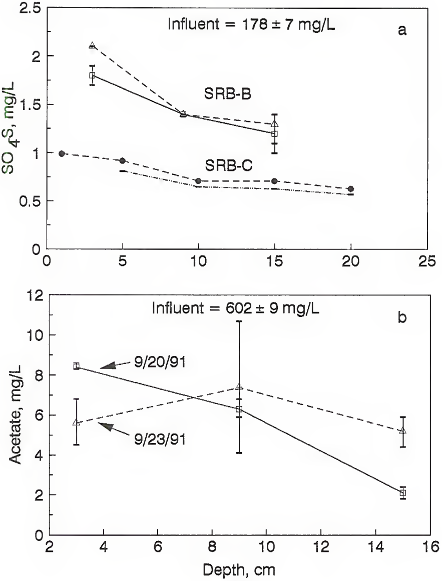


Figure 3.6. Depth profile of nonconservative elements in 4.9 and 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms a) SO_4-S; b) acetate.

Table 3.7 Depth profile of oxidation-reduction potential (Eh_7) in two 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms (n=3). No plants were in the reactors.

Depth (cm)	Oxidation-reduction potential (mV)	
	CSTR-1	CSTR-2
3	-93	-75
6	-143	-96
9	-160	-128
12	-198	-185

Table 3.8 Mean influent and effluent concentrations of water quality parameters in 2.2 and 4.9 liter continuous-flow stirred tank reactors with nitrate-reducing biofilms during four phases of operation (phases defined in Table 3.3).

Dates		Acetate		NO_3-N		C/N	SO_4-S		Eh_7	pH
		Inf	Eff	Inf	Eff	Inf	Inf	Eff		
		---------- mg/L		-----------			--- mg/L	---	- mv -	
PHASE II	NP	596.9$^+$ (76.8)	<2	167.6 (25.6)	2.5 (4.5)	1.45	12.2 (2.4)	1.7 (0.9)	126 (67)	6.6 (0.8)
	PL	629.8 (52.7)	<2	163.8 (39.2)	0.7 (1.2)	1.56	11.5 (5.3)	1.1 (0.9)	105 (66)	6.5 (0.7)
PHASE III	NP	186.9 (13.0)	2.1 (1.3)	53.5 (3.0)	2.8 (5.9)	1.42	4.0 (2.4)	1.6 (0.6)	223 (23)	6.8 (0.2)
	PL	191.4 (7.3)	1.6 (0.6)	52.6 (3.4)	5.5 (5.2)	1.48	3.8 (2.4)	1.5 (0.3)	201 (19)	6.4 (0.1)
PHASE IVa	PL	589.3 (87.9)	1.6 (1.0)	175.6 (24.3)	0.9 (2.0)	1.37	10.5 (5.2)	1.4 (1.7)	111 (94)	6.7 (0.1)
IVb	PL	621.7 (75.0)	2.0 (0.8)	173.7 (23.9)	0.8 (1.9)	1.46	9.9 (5.2)	1.1 (1.6)	141 (79)	6.3 (0.5)

$^+$Mean and standard deviation (in parentheses) for the operating time of each phase.
NP = no plants, PL = Scirpus validus
$Eh_7 = Eh_{measured} - (7 - pH_{measured}) * 59$ mv/pH unit

on removal of SO_4-S. No sulfides were detected during any phases.

Iron was added as Fe^{+2} so that Fe^{+3} would not be available as an electron acceptor. However, 40 to 73 % of the total Fe in the influent nutrient solution was in the oxidized form entering the reactor (Table 3.9). This may be due to oxidation of Fe^{+2} to Fe^{+3} by NO_3-N; a reaction which is thermodynamically favorable in an acidic solution (nutrient solution pH ranged from 2.0 to 3.0).

$$5Fe^{+2} + NO_3^- + 6H^+ \rightleftarrows 5Fe^{+3} + 1/2\ N_2 + 3\ H_2O \qquad E_{cell} = +0.47 \qquad [3\text{-}5]$$

However, greater than 60 % of the total Fe in the reactor was Fe^{+2} (Table 3.9). Therefore, there was chemical and/or biological reduction of Fe^{+3} in the nitrate-reducing reactors. Concentrations of Fe^{+2} in the reactors were less than 2 mg/L.

pH. During the first 30 days of PHASE II, both reactors (with and without plants) had rapid drops in pH (Figure 3.7a) which were remedied by raising the pH of the influent solution. When reactor pH increased above 7.5, pH of the influent was decreased to the operational influent pH = 3.0 (Table 3.5). After the initial fluctuations in pH, there was a period of about 45 days when the pH varied less than ± 0.25 pH units in both reactors. There were no significant differences between the two reactors with and without plants. The influent solution pH to both reactors was within 0.05 pH units (Table 3.5).

Table 3.9 Total and ferrous (Fe^{+2}) iron concentrations in nitrate- and sulfate-reducing continuous-flow stirred tank reactors during four phases of operation. Experimental conditions during phases are summarized in Table 3.3. Sample standard deviations are in parentheses.

	Sulfate-reducing biofilms				Nitrate-reducing biofilms			
	No plants		*S. validus*		No plants		*S. validus*	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
				------------------------- mg/L ---------------------				
PHASE I[+]								
Total Fe	138.1	17.2	137.0	6.6	--	--	--	--
	(31.5)	(16.2)	(27.6)	(4.9)				
Fe^{+2}	134.0	16.2	133.7	6.1	--	--	--	--
	(33.7)	(15.2)	(28.4)	(5.4)				
PHASE II								
Total Fe	91.9	6.0	89.2	8.0	2.5	0.7	2.6	1.9
	(24.6)	(6.6)	(24.4)	(12.2)	(0.9)	(0.7)	(1.7)	(3.1)
Fe^{+2}	81.6	5.9	81.9	7.1	0.8	0.7	0.7	1.7
	(19.1)	(6.6)	(20.9)	(11.8)	(1.0)	(1.3)	(0.7)	(3.0)
PHASE III								
Total Fe	69.7	2.4	64.3	8.3	3.0	0.5	3.1	0.7
	(5.5)	(2.8)	(10.0)	(4.1)	(0.5)	(0.4)	(0.4)	(0.7)
Fe^{+2}	65.3	2.4	61.5	8.4	1.8	0.3	1.0	0.7
	(4.8)	(2.1)	(4.1)	(3.5)	(1.1)	(0.1)	(0.6)	(0.2)
PHASE IV[♥]	C inf	C eff			F inf	F eff		
A Total Fe	122.3	9.6	--	--	13.7	0.7	--	--
	(36.8)	(7.2)			(9.0)	(0.0)		
Fe^{+2}	115.2	8.5	--	--	8.2	0.3	--	--
	(34.2)	(6.1)			(5.8)	(0.0)		
B Total Fe	99.1	10.0	--	--	5.2	2.8	--	--
	(29.3)	(8.3)			(2.2)	(2.3)		
Fe^{+2}	86.3	8.2	--	--	2.2	2.0	--	--
	(21.3)	(8.2)			(2.0)	(2.1)		

[+] No plants grown during PHASE I.

[♥] Plants were removed after 27 days

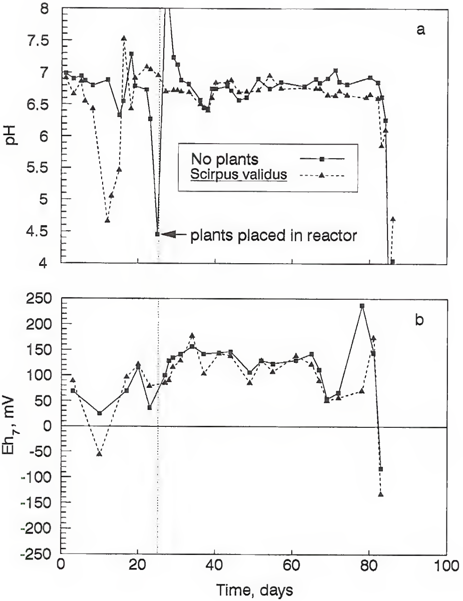


Figure 3.7. Oxidation-reduction potential and pH during PHASE II in two 2.2 L continuous-flow stirred tank reactors with nitrate-reducing biofilms. Scirpus validus was planted 27 days after start-up. a) pH; b) Eh_7.

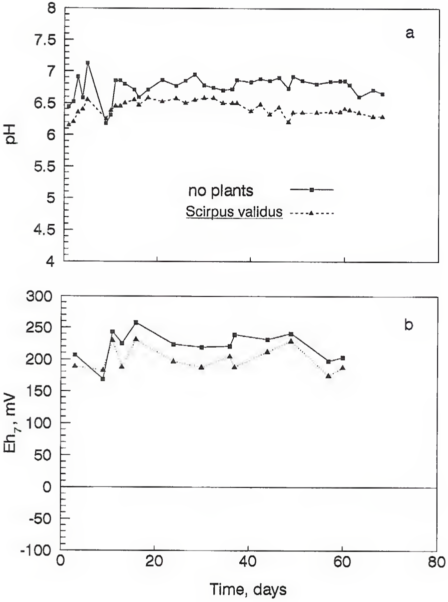


Figure 3.8. Oxidation-reduction potential during PHASE III for two 2.2 L continuous-flow stirred tank reactors with nitrate-reducing biofilms. Scirpus validus was grown for the entire period. a) pH; b) Eh$_7$.

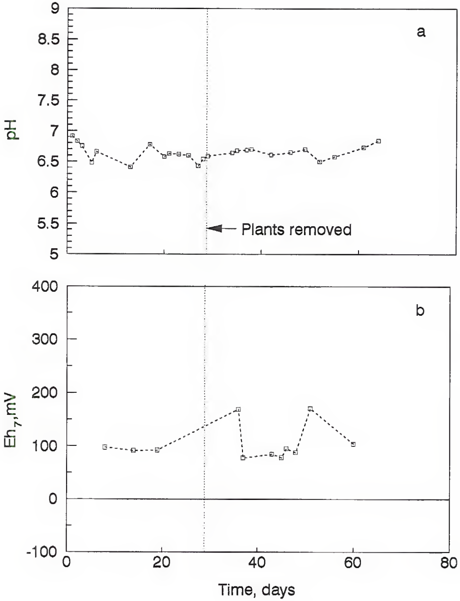


Figure 3.9. Oxidation-reduction potential during PHASE IV for a 4.9 L continuous-flow stirred tank reactor with nitrate-reducing biofilms. Scirpus validus was removed 27 days after the start of PHASE IV. a) pH; b) Eh$_7$.

During PHASE III, pH was unstable for about 15 days in both reactors. However, the pH stabilized and varied less than ± 0.25 pH units for the following 45 days (Figure 3.8a). The pH in the reactor with plants was significantly lower than in the reactor without plants. The influent pH to both reactors differed by less than 0.05 pH units (Table 3.5).

During PHASE IV, pH varied less than 0.3 pH units for about 60 days. Removal of the plants from the reactor during IV did not effect the pH (Figure 3.9a).

Oxidation-reduction potential. During PHASE II, Eh ranged from +30 to +245 mV. When the pH stabilized, Eh also stabilized. However, Eh continued to vary ± 50 mV (Figure 3.7b). There was no significant difference between reactors with and without plants. The average Eh for the two reactors with and without plants were +79 and +99 mV respectively (Table 3.8).

During PHASE III, Eh stabilized after about 15 days, after this time Eh varied less ± 50 mV in both reactors. Eh in the reactor with plants was significantly higher than Eh in the reactor without plants (Figure 3.8b). The average Eh for both reactors was significantly higher than the average Eh during PHASE II (Table 3.8). This may be related to the C and N loading rates which were 50 % lower during PHASE III than PHASE II (Table 3.7).

During PHASE IV, the Eh varied less than ± 50 mV. Removal of plants did not significantly effect the Eh in the reactor (Figure 3.9b).

Experiment IIb. Steady state sulfate-reducing cultures

C and S loading rates. Table 3.10 presents a summary of Eh, pH, acetate, SO_4-S, $S^=$, and C/S ratio for PHASEs I through IV. The stoichiometric C/S ratio for complete oxidation of acetate with sulfate was 0.75. The C/S ratios used in the reactors ranged from 0.62 to 2.10 (Table 3.10).

Acetate and SO_4-S. Effluent acetate and SO_4-S concentrations were not affected by the C/S ratio of the influent solution (Table 3.10) during PHASEs I, II, and IV. In PHASEs I, II, and IV with C/S > 1.1, greater than 98 % of the acetate and SO_4-S were removed; acetate concentrations were < 10 mg/L and SO_4-S < 5 mg/L. Figure 3.10a and b, show steady state concentrations of SO_4-S and acetate during PHASE I in two CSTRs without plants. Similar trends were also observed during PHASEs II and IV. The longer retention times during PHASE IV (Table 3.7) did not result in lower acetate concentrations.

During PHASE III, the C/S ratio was 0.62, which was less than the optimum ratio of 0.75 for complete reduction of S. The lower C/S ratio resulted in higher concentrations of SO_4-S than during other PHASEs (Table 3.10). Concentrations of SO_4-S were significantly higher in the reactors with than without plants (Figure 3.11).

Table 3.10 Mean influent and effluent concentrations of water quality parameters in 2.2 and 4.9 liter continuous-flow stirred tank reactors with sulfate-reducing biofilm during four phases of operation (Table 3.3).

		Acetate		SO_4-S		C/S	Sulfides		Eh	pH
		Inf	Eff	Inf	Eff	Inf	total	soluble		
		-----------	mg/L	------------			---- mg/L ----		- mv -	
PHASE I	NP	641.2[+]	8.0	208.0	3.3	1.25	10.4	8.6	15	6.2
		(58.1)	(2.2)	(31.3)	(3.7)		(4.6)	(6.4)	(71)	(0.2)
	NP	640.1	7.3	219.5	2.2	1.19	5.4	6.8	1	6.7
		(47.7)	(2.2)	(29.5)	(1.5)		(3.1)	(3.2)	(78)	(0.3)
PHASE II	NP	617.1	6.2	119.3	1.4	2.10	5.1	3.2	-4	6.3
		(58.3)	(2.4)	(14.1)	(0.6)		(1.6)	(1.8)	(30)	(0.3)
	PL	593.7	6.3	119.4	2.0	2.02	5.2	4.5	9	6.4
		(85.2)	(2.1)	(16.7)	(2.8)		(1.6)	(1.5)	(25)	(0.4)
PHASE III	NP	178.3	2.3	98.8	27.7	0.62	0.9	0.5	39	6.4
		(6.7)	(1.1)	(4.4)	(28.8)		(0.1)	--	(17)	(0.4)
	PL	180.3	2.1	97.2	46.8	0.62	2.8	0.9	68	6.0
		(9.7)	(1.3)	(4.7)	(22.1)		(2.5)	--	(28)	(0.3)
PHASE IV	PL	567.8	4.2	110.9	1.6	2.08	6.7	6.1	-23	6.3
		(79.9)	(2.9)	(29.2)	(1.1)		(4.3)	(3.3)	(24)	(0.3)

[+] Mean and standard deviation (in parentheses) for the operating time of each phase.
NP = no plants, PL = Scirpus validus

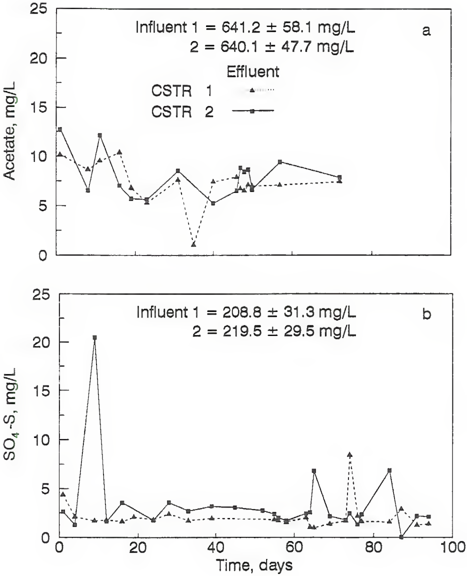


Figure 3.10. Concentration of electron donor and acceptor in two 2.2 L continuous-flow stirred tank reactors (CSTRs) with sulfate-reducing biofilms during PHASE I. No plants were present in the reactors. a) Acetate; b) SO_4-S.

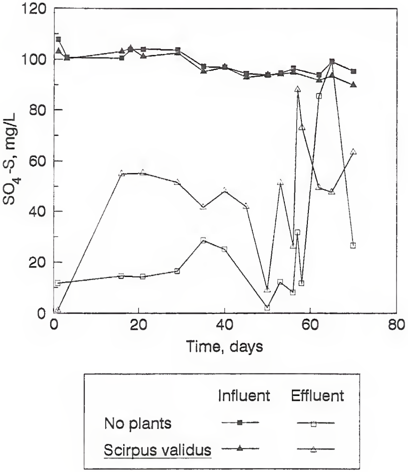


Figure 3.11. Influent and effluent SO_4-S concentrations in two 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms. Acetate loading rate was 9 mg/hr to reactors with and without plants (PHASE III).

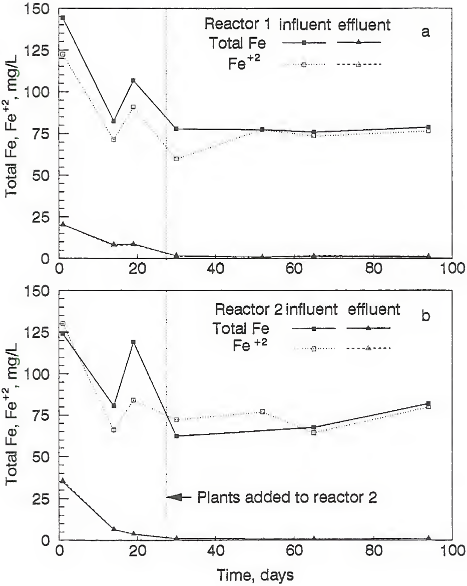


Figure 3.12. Influent and effluent concentrations of total Fe and Fe^{+2} during PHASE II. Plants were placed in reactors after 27 days. Data is representative of Fe and Fe^{+2} concentrations during PHASEs I, III, and IV.

Removal of sulfides. The ratio of influent SO_4-S/Fe^{+2} during PHASEs I, II, III, and IV was 1.5, 1.5, 1.8, and 1.1 respectively. During all phases of study the average concentrations of total Fe in the reactors was less than 8 mg/L (Table 3.9). Figures 3.12a and b show that steady state levels of Fe were less than 5 mg/L during PHASE II. The influent and effluent concentrations reached steady state once the SO_4-S/Fe ratio was established; similar conditions were also established during PHASEs II, III, and IV. Less iron was used during PHASE III than PHASE II because of the lower SO_4-S loading rate (Table 3.4).

Bacterial reduction of 1 mg of SO_4-S results in the production of one mg of $S^{=}$. The highest concentration of $S^{=}$ measured during PHASE I was 98 mg/L. Based on SO_4-S reduction, the expected sulfide concentration would be 205 mg/L during PHASEs I and II. The difference between expected and measured concentration was due to the pH of the effluent, pH > 6.9; above this pH about 50 % of the total sulfides would be soluble. Figure 3.13a shows the net effect of methods used during PHASE I to reduce sulfide levels (pH adjustment, purging the headspace with N_2 and precipitation of the sulfides with Fe^{+2}). The levels of soluble sulfides decreased from < 10 mg/L to < 3 mg/L as the phases progressed (Table 3.10). This was probably due to lower sulfate reduction rates during PHASEs II and III. The

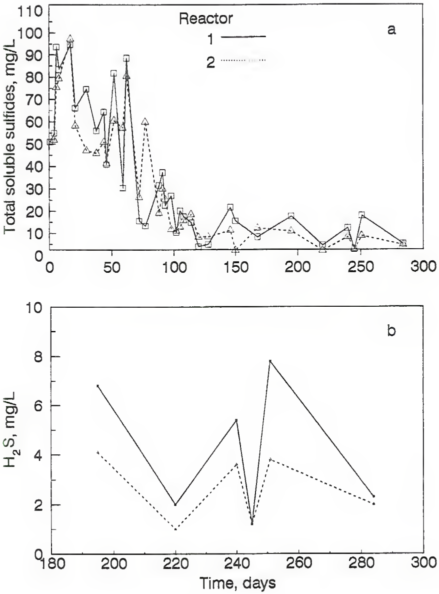


Figure 3.13. Concentration of total and soluble sulfides in two continuous-flow stirred tank reactors with sulfate-reducing biofilms. Levels of H_2S were maintained at low concentrations to reduce toxicity to Scirpus validus. a). Total soluble sulfides; b). soluble H_2S.

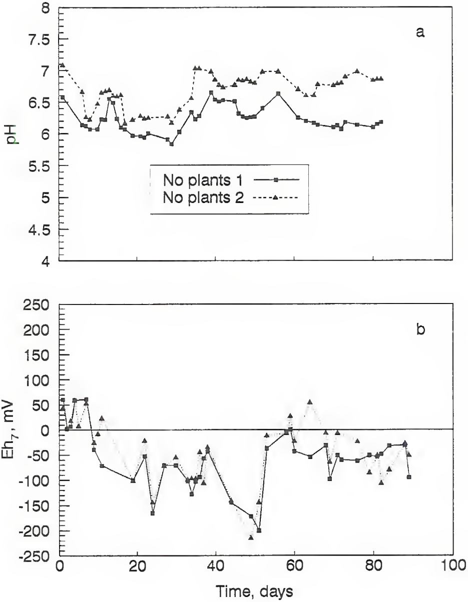


Figure 3.14. Oxidation-reduction potential and pH during PHASE I in two 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms. No plants were present in the reactors. a) pH; b) Eh$_7$.

total sulfides decreased in a similar manner (Table 3.10). The toxic form of sulfide, $H_2S_{(l)}$ was calculated based on solubility product at 25°C, at measured pH (Lawrence and McCarty 1966):

$$H_2S_{(l)} = \text{Total soluble sulfide}/[(2.28*(1+11.2^{-8})/10^{pH}))] \quad [3\text{-}6]$$

The average concentration of $H_2S(1)$ was less than 4.5, 2.2, 1.2 mg/L during PHASEs I, II, and III respectively. Figure 3.13b shows the variation of $H_2S_{(l)}$ during PHASE I.

pH. The pH of the nutrient solutions is shown in Table 3.3. Equilibrium pH in the reactors is shown in Table 3.10. Reactors receiving the lowest C loading rates (Table 3.7) required the least acid in the nutrient solution to maintain the pH of the reactors (Table 3.3).

During PHASE I, pH varied about one unit at the beginning of the study, and less than ± 0.25 units for the later half of the study (Figure 3.14a). There were significant differences between the two reactors without plants. Operation of the two was identical except that influent pH to reactor 1 was higher than in 2. This kept the pH of reactor 1 in a range suitable for the bacteria. Although influent pH to 1 was higher than 2, reactor pH in 1 was lower.

During PHASE II, the pH in both reactors varied less than ± 0.25 pH units for the first and last 25 days (Figure 3.15a). Prior to introduction of plants there were no significant differences in pH between the two reactors.

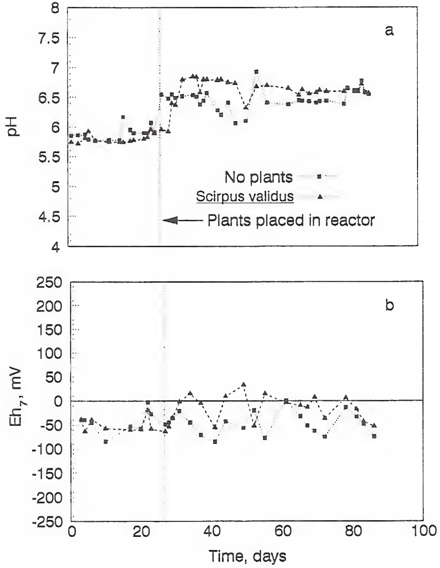


Figure 3.15. Oxidation-reduction potential and pH during PHASE II in two 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms. Scirpus validus was planted 27 days after start-up. a) pH; b) Eh_7.

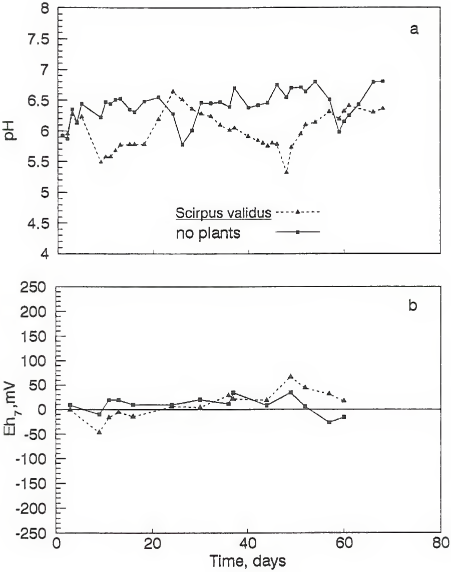


Figure 3.16. Oxidation-reduction and pH during PHASE III in two 2.2 L continuous-flow stirred tank reactors with sulfate-reducing biofilms. Scirpus validus was planted for the entire period. a) pH; b) Eh_7.

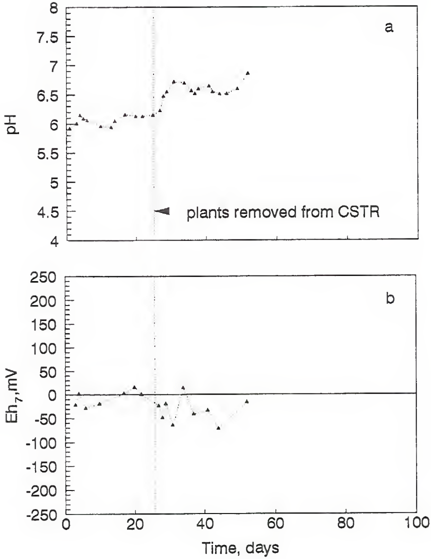


Figure 3.17. Oxidation-reduction potential and pH during PHASE IV in a 4.9 L continuous-flow stirred tank reactor with sulfate-reducing biofilms. Scirpus validus was removed 27 days after start-up. a) pH; b) Eh$_7$.

After placement of plants in the reactors the pH was significantly higher than in the reactor without plants. During PHASE III, the pH was significantly lower in the reactor with than without plants (Figure 3.16a). The pH in the reactor without plants was generally stable at about 6.4, and varied less than ± 0.5 pH units. When both reactors received nutrient solution with the same pH, the pH in the reactor with plants fell below 5.5 (Figure 3.16a) which disrupted the culture stability. There was considerable variation of pH in the reactor with plants, because the pH of the nutrient solution was adjusted whenever the pH in the reactor dropped below 5.5.

The reactor was operated for 30 days with plants during PHASE IV. When plants were removed, pH increased significantly by about 0.5 pH units (Figure 3.17a).

Redox potential. Eh was unstable in both reactors during PHASE I (Figure 3.14b). Although no plants were present in the reactors, there was a small but significant difference in Eh between the two reactors; Without statistics the differences would not be obvious.

During PHASE II, Eh was more stable in both reactors than during PHASE I, varying from -100 to +50 mV. Placement of plants in one reactor after 27 days caused a significant increase in Eh (Figure 3.15b). There was a significant difference between reactors with and without plants, for a period of 45 days after placing plants in the reactor.

Redox potential varied from -50 to +50 mV during PHASE III (Figure 3.16b). There were no significant differences between the reactors with and without plants.

During PHASE IV the Eh varied from -90 to +10 mV. There was a small but insignificant decrease after plants were removed from the reactor (Figure 3.17b).

Summary of effect of plants on pH and Eh. During PHASEs III and IV plants caused a significant decrease in pH in nitrate- and sulfate-reducing reactors with plants (Table 3.11). During PHASE II in the NRB and SRB reactors, the pH in reactors with plants was either equal to or greater than pH without plants.

There were no significant differences in Eh_7 between the NRB reactors with and without plants during PHASEs II and IV. During PHASE III the Eh_7 was lower in the reactor with plants.

Plants in the SRB reactors caused a significant increase in the Eh_7 during PHASE II and IV (Table 3.11). This effect was noted when plants were removed (PHASE IV) or when they were placed in the reactor and compared to a reactor without plants (PHASE II). However during PHASE III, the plants did not have a significant effect on the Eh_7.

Total solids. The best measure of total solids in the reactors was achieved by stirring the contents of the reactor, removing the solids, and drying them at 80°C for 24 hours. Subsampling the Tri-pak media in the reactors

Table 3.11 Summary of plants effects on pH and Eh in nitrate-and sulfate-reducing continuous-flow stirred tank reactors during four phases of operation. Analysis of variance (SAS 1987) compared mean differences between treatments with and without plants, or before and after plants were placed in reactors. Acetate, SO_4-S, and NO_3-N concentrations were not effected by plant treatments.

	PHASE		
	II	III	IV
Nitrate-reducing			
Eh_7	PL = NPL	PL < NPL	PL = NPL
pH	PL = NPL	PL < NPL	PL < NPL
Sulfate-redcuing			
Eh_7	PL > NPL	PL = NPL	PL > NPL
pH	PL > NPL	PL < NPL	PL < NPL

PL = Scripus validus
NPL = no plants

underestimated the total amount of solids. Total solids accumulation rate was highest during PHASE I when reactors received the highest concentration of Fe (Table 3.9 and 3.12). Due to the high iron loading rates, accumulation of total solids was much greater in the sulfate than in the nitrate-reducing reactors (Table 3.12).

Bacterial solids. The rate of accumulation of bacterial solids was higher in nitrate-reducing reactors than in sulfate-reducing reactors (Table 3.12). There were also higher concentrations of bacteria in suspension in nitrate-reducing reactors. The solids in suspension made it more difficult to filter water samples through 0.45 μm filters.

The standard method of ashing oven-dry solids and determining the bacterial fraction (APHA 1989) was not applicable for oven-dry solids from the sulfate-reducing reactor. This was because the solids underwent mineralogical changes indicated by a change from dark black color (oven-dry solids) to reddish brown after ashing. Change in mineralogy of the oven dry and ashed solids was verified using a Nicolet X-ray diffractometer. Characteristic D-spacing for the diffraction peaks were identified using a mineral powder diffraction file (JCPDS 1980). The oven-dry solids were almost 100% pyrite (Fe_2S), after ashing they were almost 100% hematite (Fe_2O_3). Therefore, change in weight after ashing was due to loss of

Table 3.12. Accumulation rates of total and bacterial solids+ in continuous-flow stirred tank reactors (CSTRs) with nitrate- and sulfate-reducing biofilms during four phases of operation.

Reactor type	Growth period	Total solids	Bacterial Solids
	--- days ---	-------- mg/day	------
SULFATE-REDUCING CSTR			
PHASE I			
NPL	174	176.4	16.2
PL	174	300.6	27.6
PHASE II			
NPL	216	154	14.2
PL	216	138	12.7
PHASE III			
NPL	72	129.2	7.4
PL	72	137.5	9.8
PHASE IV			
PL	189	45.5	5.9
NITRATE-REDUCING CSTR			
PHASE II			
NPL	190	27.1	14.7
PL	192	38.5	22.5
PHASE III			
NPL	97	34.0	19.3
PL	97	44.3	21.4
PHASE IV			
PL	189	18.9	11.7

+Calculation of bacterial solids for sulfate-reducing reactors was based on C and N analysis of total solids. Calculation of bacterial solids for nitrate-reducing reactors was based on weight change in the total solids after ashing at 500°c.

NP = control reactor without plants,

PL = reactor with _Scirpus validus_

bacterial biomass and oxidation of Fe. Stoichiometric oxidation of FeS_2 to Fe_2O_3 should result in 33% weight loss. Therefore any weight loss greater than 33% of the dry solids, should be due to volatilization of bacterial biomass. Unfortunatley, this approach was unreliable because the net weight change after ashing oven-dry solids was often less than 33 %.

Oven-dry solids from SRB reactors contained from 3.8 to 8.5 %C and 0.5 to 1.3 %N; oven dry solids from NRB reactors contained 21.4 to 28.5 %C, and 3.0 to 4.8 %N. The lower %C and %N content of solids from the SRB reactors was due to the high content of FeS_2. Average %C and %N content were used to calculate bacterial biomass in the oven dry solids.

Bacterial solids accumulation rates in the SRB reactors decreased during PHASEs II and III (Table 3.12) when C and S loading rates were lower (Table 3.7). There was no apparent effect of plants on accumulation of solids.

Discussion

Design and Hydraulic Characterization of CSTR

Homogeneity

Results from dye studies showed that the reactors are completely mixed, and function as continuous-flow stirred tank reactors. Sulfate and acetate concentrations showed minimal variations with depth. Differences with depth were less than 1% of the influent concentration for both SO_4-S and acetate. Using a similar reactor configuration, Rittman et al. (1986) observed a difference of 6% of the influent concentration in packed columns they considered to be completely mixed.

Steady state concentrations

Concentrations of electron donors and acceptors verified complete mix assumptions; however Eh profiles were stratified with depth. The apparent stratification may be due to local variations in Eh within the biofilm. During measurments of Eh at the 3 cm depth, the tip of the platinum electrode was in the recycle flow above the plastic media. Therefore, the measurement represents Eh of the fluid after passing through the entire depth of the reactor. When taking measurements at lower depths (6, 9, 12 cm), the electrode was forced down into the biofilm covered Tri-pak plastic media. Therefore, the Eh measured may have been a measurement within the bacterial biofilm. Sulfide precipitates within the biofilms may also have interfered with the Eh measurements (Whitfield 1974).

A goal in designing a CSTR was to create both homogenous and steady state conditions. Both conditions facilitate sampling and are fundamental to the mass balance used to estimate O_2 transport. If these conditions are not met, sampling from the reactor headspace at any given time will not be representative of the entire reactor for any other time. In an ideal CSTR, biological and chemical reactions should be in steady state. Concentrations of electron donor and acceptors, Eh, and pH, were used to determine steady state. Electron donor and acceptors were consistently removed at efficiencies greater than 95% (Table 3.8 and 3.10) in all NRB and SRB reactors during phases I, II and IV, and in NRB reactors during PHASE III. At these high removal rates the effluent concentrations were low and relatively constant. Although the coefficient of variation for effluent concentration of electron donors or acceptors was often 100%, the concentrations were generally less than 1 % of the influent. Therefore, based on effluent concentrations of SO_4-S, NO_3-N and acetate, reactors were both homogenous and at steady state.

Establishment of steady state was tenuous during PHASE III in the SBR reactor with plants. The reactor without plants was relatively steady during the same period (Figure 3.11). In the reactor with plants, coefficients of variation for effluent SO_4-S were only about 50%, however this variation was large relative to the influent concentration. This variability might have been resolved if the reactors had been operated at a higher pH.

Steady state Eh and pH

Whitfield (1974) observed that Eh measured with a platinum electrode should be reliable within ± 50 mV. Therefore, I assume that variation of Eh within this range was an artifact of the technique, and was therefore representative of steady state. Despite some periods of instability during PHASE II, III, and IV, the Eh varied less than ±30 mV in all NRB (Table 3.8) and SRB reactors (Table 3.10). In general, Eh was most stable during lower acetate loading rates. Therefore relative to Eh, the reactors were in steady state redox environments.

There were periods of large decreases in pH in both the NRB and SRB reactors. Stability of pH depends on the neutralization of influent acidity with bicarbonate produced from acetate oxidation; pH decreases when oxidation of acetate decreases. Drops in pH were assumed to be due to changes in the ability of the bacteria to oxidize acetate. However, increases in concentrations of electron donors and acceptors were generally not observed when pH decreased. The biogeochemical reactions in the reactors are complex, with the available data the factors influencing culture stability cannot be clearly identified. Further research is required to determine what factors influence the stability of the cultures.

Despite periodic changes, the pH varied less than ± 0.25 pH units for atleast 30 days in all reactors during all phases, except for the SRB with plants during PHASE III (Figures 3.10 to 3.16). Therefore, steady state pH (variation < 0.25 pH units) was maintained in the reactors for relatively long periods of time.

Bacterial environment

Another property of an ideal steady state is that rate of change of bacterial biomass should equal zero, that is death and decay should equal production (Grady and Lim 1980). Although bacterial accumulation rates were low (Table 3.12) they were not zero. This should not disrupt the mass balance since acetate incorporated in biomass was accounted for. During the low loading rate (PHASE III) close to 10% of the acetate was incorporated into bacterial biomass.

In chemostats, bacterial suspensions are continuously stirred to maintain complete-mix conditions (Grady and Lim 1980). Biofilms generally consist of layers of bacteria. In a complete-mix environment, only bacteria on the surface of the biofilm are exposed to the homogenous environment. Therefore, the reactors consist of two distinct zones (i) water in the macropores (which was completely mixed), and (2) water within the biofilm and root mat. This second region is dominated by diffusive flux of nutrients, which will result in heterogenous conditions. Although complete-mix conditions exist for the pore water the zone in and around the roots and biofilm may be heterogenous. I have assumed that these two regions are in equilibrium.

Control of Eh and pH

The control of Eh and pH in the reactors depends on maintaining anaerobic conditions, and proper addition of acid and nutrients to the influent. No special instrumentation was required for pH control since bicarbonate produced during acetate oxidation neutralizes the acidity of the nutrient solution:

$$CH_3COO^- + SO4^{2-} + 3/2\ H^+ \leftrightharpoons HCO_3^- + 1/2\ HS^- + 1/2\ H_2S + H_2O + CO_2 \qquad [3\text{-}7]$$

$$5/8\ CH_3COO^- + NO3^- + H^+ \leftrightharpoons 5/8\ HCO_3^- + 9/8\ H_2O + 5/8\ CO_2 + 1/2\ N_2 \qquad [3\text{-}8]$$

Ancillary benefits of an acidified nutrient solution are the inhibition of algal and bacterial growth, lower rate of oxidation of Fe^{+2} (Benefield et al. 1982), and less precipitation of Fe^{+2} complexes in the SRB nutrient solution containers. The loading rate of electron acceptors and donors, and target pH determines the amount of acid required to neutralize the HCO_3^- produced during oxidation-reduction reactions. Table 3.5 shows the different amounts of concentrated HCL that were required to maintain steady state pH at any given acetate loading rate (Tables 3.8, and 3.10). Steady state Eh is a function of pH (Equation [3-2]). The steady state Eh was attained by controlling input of specific electron donors and acceptors, adjustment of the nutrient solution pH, and maintenance of anaerobic conditions.

Methods for controlling pH and Eh were mechanically simpler than methods used in controlled Eh and pH batch reactors developed by Patrick et al. (1973) and Reddy et al. (1976). Establishment of steady state Eh and pH in the reactors does not require continuous monitoring of Eh or pH, consequently no instruments or controllers are needded for turning on air pumps and dosing equipment for acid or base. Besides being simpler mechanically, the reactors provide a steady state environment, which is not possible in batch reactors.

In experiments using controlled Eh batch reactors (Masscheleyn et al. 1990; 1991; Olila 1992; Patrick et al. 1973), Eh was defined by a voltage output which was presumed to imply specific redox conditions and bacterial processes (Table 3.1). In the reactors presented here Eh is not used as a control parameter. It simply represents the voltage output due to a mixture of bacterial activity dominated by nitrate and sulfate reduction, and methanogenesis. This approach to Eh and pH control was simple and effective. Future research should be able to use these CSTRs to study effects of anaerobiosis and pH on plants and bacteria in anaerobic environments.

The Nernst equation ([3-2]) shows that Eh is a function of pH and activities of oxidized and reduced species in solution. Use of the Nernst equation to calculate an expected Eh based on measured pH and concentrations of chemical species (activity was assumed to equal unity) resulted in Eh values very different from those measured in the reactors (Figures 3.18 and 3.19), and from those

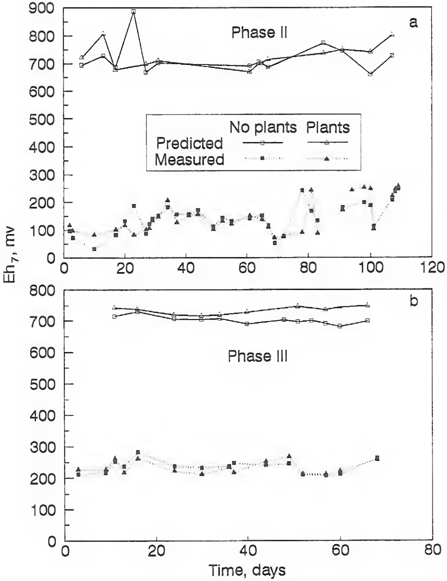


Figure 3.18. Comparison of measured and predicted Eh during two acetate loading rates in nitrate-reducing reactors. Predicted values were calculated using the Nernst equation solved with measured concentrations of NO_3-N, atmospheric partial pressure nitrogen, and pH. a) PHASE II; b) PHASE III.

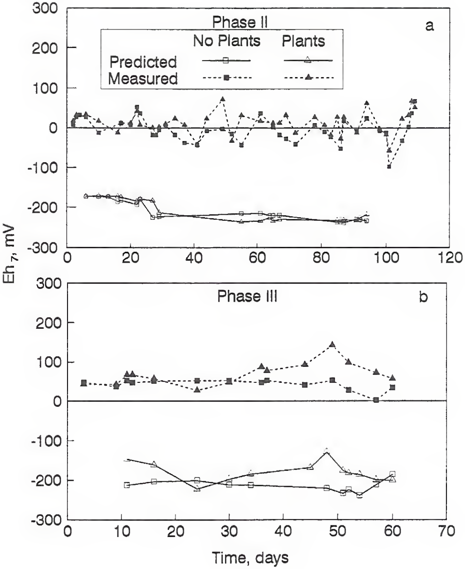


Figure 3.19. Comparison of measured and predicted Eh values for two acetate loading rates in sulfate-reducing reactors. Predicted values were calculated using the Nernst equation solved with measured concentrations of SO_4-S, HS^{-1}, H_2S, and pH. a) PHASE II; b) PHASE III.

measured values in the SRB reactor (Figure 3.19) but significantly higher than the measured Eh values in the NRB reactors (Figure 3.18). Differences between measured and calculated Eh values is not uncommon in work on natural systems (Holm and Curtiss 1989; Jackson and Patterson 1982). However, some field data for the SO_4^{2-}/S^{2-} redox couple has been correlated well with calculated potentials (Whitfield 1969). The Eh measurements do agree with other field observations; Takai and Kamura (1966) reported SO_4-S reduction in waterlogged soils with Eh values ranging from 0 to -190 mV.

Stumm (1966) noted that Eh in natural systems cannot be adequately predicted by the Nernst equation because of multiple competing redox couples. Although SO_4-S and NO_3-N were the primary electron acceptors in the CSTRs, there were several competing redox couples in the reactors. Methanogenesis was confirmed in the SRB reactors during PHASEs I, II and IV. It is also likely that small amounts of O_2 leaked through the Norprene tubing used in recycle lines for both sets of reactors. Reactors contained a mixture of electron donors (acetate, NH_4^+, S^{2-}, Fe^{+2}) and acceptors (O_2, SO_4^{2-}, NO_3^-, and Fe^{+3}) which make Eh difficult to interpret.

Plant growth and environmental factors

This study has shown that anaerobic sulfate-reducing and methanogenic microcosms can be designed for the growth of emergent aquatic plants. Although growth of S. validus was stunted (relative to plants in their native habitat), they did continue to produce new tillers and root biomass during the study period. No published research was found on the toxicity of sulfides to Scirpus validus. Sulfides were maintained at levels less than 5 mg/L; however, they could be controlled at any level (5-90 mg/L in this experiment). It was possible to maintain sulfides at concentrations that have been shown to be non-toxic to the freshwater plant Panica hemitomon and the salt water marsh plant Spartina alterniflora (Koch et al. 1990). At sulfide concentrations of 32 mg/L and above, root energy status, NH_4^+ uptake and leaf growth were negatively affected in the fresh water macrophyte Panicum hemitomon (Koch et al. 1990). Allam and Hollis (1972) found inhibition of oxidases in Oryza sativa at concentrations less than 1 mg/L $H_2S_{(l)}$. Plant growth was also stunted in the nitrate-reducing reactors. The poor plant growth may have been due to the low light levels (170 μeinsteins/cm^2 sec). Future studies in a greenhouse with mature plants will help evaluate effects of environmental stresses on plants.

Research has shown that Fe^{+2} may be toxic to plants and will form visible FeOOH plaques on Typha latifolia roots at concentrations as low as 5 mg/L Fe^{+2} (Taylor et al. 1984); and at 10 mg/L plaque formed on roots of Oryza sativa (Green and Etherington 1977). This was suspected to diminish the plants ability to absorb essential nutrients (Howeler 1973). A SO_4^{2-}/Fe^{2+} ratio ranging from 1.1 to 1.5 kept both $S^{=}$ and Fe^{+2} concentrations lower than 5 mg/L. Although Fe^{+2} concentrations were low, the roots of plants in the SRB reactors were thoroughly coated with a black film, presumably pyrite, FeS_2. Concentrations of S^{-2} and Fe were so low that they were of no concern to plant health in NRB reactors.

Comparison to natural and constructed wetland systems

Wetlands ecosystems have active nitrate- and sulfate-reducing, and methanogenic populations at various soil depths depending on the availability of electron acceptors. Constructed wetlands have been designed for removal of nitrate from wastewater effluent. Nitrate removal rates for these systems have ranged from 0.01 to 0.02 g N/m^2 day in organic soils (natural wetland) and from 0.10 to 0.82 g N/m^2 day in mineral soils (constructed wetlands)(Gale et al. 1993). Nitrate removal rates in the CSTRs ranged from 13 to 44 g N/m^2 day. The SO_4-S loading and removal rates were also much higher than in natural systems. Pore water in estuarine wetlands, and wetlands used for treatment of acid mine drainage are both high in SO_4. SO_4 reduction rates measured in estuarine wetlands were 1.6 g S/m^2 day (Howes et al. 1984), and in acid mine drainage wetlands were 0.14 to 2.41 g S/m^2 day (Herlihy and Mills 1985). Sulfate reduction rates in the CSTRs ranged from 14.0 to 28 g S/m^2 day.

Carbon oxidation rates measured in an estuary ranged from 1.0 to 2.2 g C/m^2 day (Howes et al. 1984; Michelson et al. 1989); oxidation rates in the CSTRs ranged from 0.46 to 1.45 g C/m^2 day. Influent concentrations of Fe and SO_4-S were in a similar range as influent to acid mine drainage wetlands which receive up to 330 mg/L SO4-S and 220 mg/L Fe (Kleinmann and Girts 1987). Since loading rates to the CSTRs are adjusted simply by changing the concentrations in the nutrient solution, and/or inflow rates, these reactors can be used to simulate a range of conditions in both natural and constructed wetlands.

Acetate is produced during the fermentation of organic material. Oxidation of acetate may account for 70 % of the SO_4-S reduction and methanogenesis in freshwater sediments (Lovely and Klug 1986). This implies that use of acetate as an electron donor is appropriate for simulating anaerobic bacterial ecology in freshwater sediments. Pfennig (1989) reported that sulfate-reducers from freshwater environments lack the capacity to oxidize acetate; however, acetate-oxidizing sulfate-reducing bacteria have been found in polluted freshwater environments (Zaiss 1984). Although acetate was the electron donor chosen in this study, any electron donor could have been supplied.

Wetland simulation without soil?

An obvious and important difference between these reactors and a natural system (and reactors developed by

Patrick et al. 1973) is the lack of soil. The CSTRs were designed to simulate biogeochemical processes in wetland soil. However, the diverse and undefined mixture of electron donors and acceptors found in a soil complicate C mass balances. The organic composition of soil in a batch reactor is constantly changing; easily oxidized coumpounds are depleted first, then residual humic and fulvic acids accumulate. Therefore, it is impossible to establish a steady state, which facilitates studies with bacterial competition, nutrient cycling, or O_2 transport. The diminishing availability of electron donors and acceptors which occur in batch systems with soil, also cause the bacterial populations to go through nonsteady growth cycles.

The use of biofilms in a CSTR without a soil suspension also has several advantages. Samples are low in suspended solids and are easily filtered (all samples were filtered through 0.45 μm filters) and analyzed. Supplying external sources of C and N with an inert media make it possible to do mass balance on the bacterial ecosystem being studied.

These CSTRs could be designed for studying biogeochemical processes in soil suspensions. This would allow studying redox proceeses in anaerobic environments, without occasional pulses of O_2 characteristic of the current reactor design (Patrick et al. 1973).

Conclusions

This research has presented a new design for simulating anaerobic wetland environments. The reactor is a continuous-flow stirred tank reactor (CSTR) with anaerobic biofilms growing on an inert plastic substrate. Complete-mix hydraulics were verified using a conservative tracer (Rhodamine WT) and depth profiles of nonconservative elements in the reactor. Although the reactor was hydraulically completely-mixed, it is probable that inside the biofilm and root mat complete-mix conditions did not exist.

Two types of anaerobic environments were simulated, (i) a sulfate-reducing and (ii) nitrate-reducing biofilms. Bacterial communities were selected for by adding specific electron acceptors (SO_4-S and NO_3-N) and donor (acetate) to reactors maintained under O_2 free conditions. At an acetate loading rate of 30 mg/hr, methanogens competed with the SRB for the electron donor. At an acetate loading rate of 9 mg/hr, no methanogens were detected in the SRB reactor. Methanogens were not detected in the NRB reactors at either acetate loading rate.

Steady state conditions in the reactors were confirmed using Eh_7, pH, SO_4-S, NO_3-N and acetate. Variations of ± 50 mV in Eh, and ± 0.25 pH units were considered to be in steady state. By this definition, Eh and pH were maintained at steady state levels for atleast 40 days in both types of reactors. Eh is a function of the pH, and activities of oxidized and reduced species in solutions. Steady state Eh was maintained by (i) controlling the pH, (ii) fixed C/N and C/S ratio determined the final concentration of oxidized and reduced species, and (iii) reduced S was precipitated with Fe^{+2}. No external meters or dosing devices were needed to "control" the Eh and pH. This method for controlling Eh and pH is mecahnically simpler than methods currently used for studying redox processe in anaerobic environments.

Growth of plants in a SRB reactor is complicated by the production of $H_2S_{(l)}$ which is toxic to plants and bacteria. Concentrations of S^{-2} and Fe^{+2} were maintained at levels (<5 mg/L) which were nontoxic to the plants. However low light levels may have restricted growth.

Reduction rates of SO_4 and NO_3 were higher than found in natural and constructed wetlands. However more realistic rates can be established simply by changing influent concentrations and/or flow rate. The reactors offer an alternative to the current method which uses batch soil suspensions for simulating anaerobic wetland environments. Although the CSTRs developed do not contain a soil they offer several advantages over the batch method with soil. These are i) Eh is controlled by steady state chemical and bacterial processes found in nature, ii) no electrical and mechanical instruments are needed to "control" the Eh and pH, and iii) O_2 was not introduced into the anaerobic environment.

Further research is needed to improve the utility of the design. The design has many potential uses, a few possibilities are: studying degradation of agricultural pesticides in runoff, nutritional requirements of aquatic plants in anaerobic environments, bacterial interactions in the wetland rhizosphere, and bioremediation in anaerobic environments.

CHAPTER 4

ESTIMATION OF O_2 TRANSPORT THROUGH SCIRPUS VALIDUS BASED ON OXIDATION OF CARBON AND NITROGEN IN ANAEROBIC WETLAND MICROCOSMS

Introduction

Emergent aquatic plants growing in constructed wetlands have the ability to sequester N and P in plant biomass and transport O_2 into the rhizosphere. These two mechanisms may have significant effects in removal and oxidation of C and N in wastewater applied to constructed wetlands (Reed et al. 1988; EPA 1989; Reddy et al. 1989).

The amount of O_2 which is available to bacteria in the rhizosphere has not previously been quantified, but needs to be (Cooper 1990). Quantification will facilitate development of engineering design guidelines for C and N removal in wetlands (Reed and Brown 1992). There have been two basic approaches for quantifying O_2 transport through aquatic plants: (i) a direct method which measures accumulation of O_2 in the root zone or solution due to plants, and ii) an indirect method which infers O_2 transport by comparing removal of BOD or NH_4-N in experimental units with and without plants (as in Chapter 2). The differences in C and N oxidation in reactors with or without plants, are related to O_2 transport using stoichiometric relationships.

Direct measurement of O_2 release from roots is difficult to quantify in a natural environment. The consortia of aerobes and anaerobes in the rhizosphere represent an immediate sink and possibly a driving force for O_2 diffusion from the root. In experiments by Armstrong et al. (1990) and Armstrong and Armstrong (1998; 1990) natural sinks for O_2, such as bacteria and reduced compounds (i.e. Fe^{+2}, S^{2-}), are removed from the rhizosphere; therefore, O_2 accumulates and can be measured. Laboratory research on O_2 transport often depends on use of gold (YSI O_2 meter) or platinum electrodes for detecting O_2 in a solution free of soil and bacteria (Moorhead and Reddy 1990; Reddy et al. 1989; Armstrong and Armstrong 1988, 1990; Armstrong et al. 1988, 1990). Measurement of O_2 transport in natural anaerobic environments with platinum or gold electrodes is complicated by several factors. The electrodes may be poisoned in a sulfate-reducing environment (Whitfield 1972), and O_2 is immediately consumed in bacterial respiration. Loss of natural concentration gradients between the roots and nutrient solution may reduce the net release of O_2 (Armstrong et al. 1990). Therefore, Armstrong et al. (1990) designed a flow cell which constantly flushes deoxygenated water past the plants to simulate soil O_2 demand.

The indirect method may be used in the laboratory or field; however, it is most effective in the field with mature plants and established bacterial associations in the rhizosphere. Use of the indirect method in field studies (Chapter 2) showed a significant effect of plants on C and N removal (not due to plant assimilation). However, the estimated O_2 transport rates were different depending on whether removal of C or N was used for the calculations.

The difference in O_2 transport rates using the indirect method may be due to limitations in accurately accounting for all respiratory pathways in the rhizosphere. Another drawback with field scale systems is the inability to control environmental parameters which influence O_2 transport, and to quantify oxidation of C and N due specifically to O_2 transport through the plants.

Wetland plants have unique adaptations to life in the anaerobic environment. A porous spongy tissue, arenchyma, which develops in wetland plants can facilitate movement of air into the roots (Conway; 1937; Van Raalte 1941; Teal and Kanwisher 1967; Armstrong 1978). It was initially thought that gas transport within the wetland plants was due to molecular diffusion. However, research has indicated convective gas flows as the major transport mechanism (Dacey 1980; Armstrong et al. 1988; Brix 1988; Grosse 1989). A theory of thermo-osmotic pressure differential has been suggested by Grosse (1989) to explain the sunlight dependent convective gas flows. The theory has been substantiated by the measurement of convective flows of gases, and differentials in pressure and partial pressure of gases within emergent aquatic plants (Brix 1988; Armstrong and Armstrong 1990). Convective gas flows are reduced at nighttime, a time when diffusion of air may be more significant in providing air to the roots (Armstrong and Armstrong 1990).

The most comprehensive studies on O_2 transport through an aquatic plant have been done on Phragmites australis (Armstrong and Armstrong 1988, 1990; Armstrong et al. 1988, 1990). Although there is clear evidence of O_2 transport into the roots by diffusion and mass flow, O_2 transport may at times be insufficient to meet the plants metabolic requirements (Brix 1990). Fermentative respiration is a second mechanism plants have adapted to provide energy for root respiration (Smith and Rees 1979). It is inefficient relative to aerobic respiration; only two ATP are provided during fermentative respiration versus 36 in aerobic respiration. Alcohol dehydrogenase (ADH) is the last enzyme in the fermentative pathway and its level in roots can be used as an indicator of fermentative respiration (Pedrazzini and McKee 1984). Research has shown that many wetland plant species are capable of switching to fermentative respiration. During long periods of inundation plants may develop arenchyma and reduce their dependence on fermentative respiration (Pedrazzini and McKee 1984; McKee et al. 1989). Fermentative respiration implies that roots are not receiving sufficient O_2 for aerobic respiration.

McKee et al. (1989) found that three plants commonly used in constructed wetlands for waste treatment have different responses to anaerobic root conditions. The cattail, Typha glauca, showed no significant changes in ADH levels in root tissue when water levels were changed. However, longstem bulrush, Scirpus validus, and common reed, P. australis, both showed increased ADH production within a month of flooding, followed by a decrease in ADH levels after six months. In comparisons of C and N removal from wastewater among these three species, S. validus was most effective (Gersberg et al. 1983; 1984; 1986). Carbon and N removal were not due to plant assimilation and are presumed due to O_2 transport through the plant (Gersberg et al. 1983; 1986). However, after 13 months in anaerobic conditions S.validus, P. australis and T. glauca maintained detectable levels of ADH in root tissue. The ADH levels in the roots were related to the Eh of the soil (McKee et al. 1989). This implies that O_2 demand by the roots is related to soil Eh. Current theories on O_2 transport do not consider the anaerobic conditions of the rhizosphere. All curently accepted mechanisms involve forces acting on the above ground portion of the plant (e.g., sunlight, humidity and venturi effect of wind (Armstrong and Armstrong 1990), which induce convective flows into the root zone.

The reactors designed in Chapter 3 will be used to estimate oxygen transport using the indirect method. The advantage these reactors have over field experiments is that environmental parameters (Eh, pH, temperature) and the availability of electron donors and acceptors can be controlled; natural bacterial populations are also maintained.

The objectives of this study were to estimate O_2 transport through Scirpus validus grown in nitrate-reducing and sulfate-reducing biofilm reactors (NRB and SRB respectively) by (i) mass balance on oxidizable C, and (ii) oxidation of $^{15}NH_4$-N. The hypothesis is that O_2 transported into the root zone is in excess of the roots metabolic requirements, and is available for oxidation of C and N by hetero- and autotrophic bacteria in the rhizosphere. Flux of excess O_2 through roots is also hypothesized to be independent of the type of anaerobic bacterial community functioning in the wetland.

Materials and Methods

Theoretical Basis for Mass Balance on Acetate

Oxygen transport was estimated using a mass balance of electron donors and acceptors in the NRB and SRB reactors.

Oxygen transport through the plants was estimated by accounting for the dominant oxidative pathways of acetate in reactors with and without plants. The difference between the acetate mass balances for reactors with and without plants is assumed to be due to O_2 transport through plants. Anaerobic respiratory pathways in nature are diverse and complicated; therefore, simplifying assumptions about the anaerobic biofilms were made to facilitate the mass balance.

Nitrate-reducing biofilms

A diverse consortium of anaerobes are involved in the partial or complete reduction of NO_3 to N_2 (Tiedje 1988; Stouthamer 1988). This research assumes that complete denitrification is the dominant pathway in NO_3 reduction. The most common dentrifiers in nature, *Pseudomonas*, and *Alcaligenes* are both capable of complete denitrification (Tiedje 1988). Both genera have also been indicated as dominant bacteria in wastewater denitrification (McCarty et al. 1969; Monteith et al 1980; Yoshida and Hirata 1981). The seed material for the NRB biofilms was secondary wastewater from the University of Florida wastewater treatment plant. Figure 4.1 shows the predominant oxidative pathways for acetate in the NRB reactors. Pathways for acetate oxidation accounted for were NO_3, SO_4, and Fe^{+3} reduction, methanogenesis, and bacterial growth. Concentrations of total Fe and SO_4 were added at low levels to meet the requirements of the plants and microbes.

Although concentrations were low (<5 mg/L) in the NRB, they were accounted for in the acetate mass balance.

Sulfate-reducing biofilms

There are only a few species of freshwater SO_4 reducing bacteria which use acetate as an electron donor. *Desulfotomaculum acetoxidans* is the most common SO_4 reducing, acetate oxidizing bacteria found in wastewater (Widdel 1988). Seed material for the SRB reactors was primary wastewater effluent from the University of Florida wastewater treatment plant. Complete reduction of SO_4 to S^{2-} is common for most sulfate-reducing bacteria (Widdel 1988). Yeast extract provided required but unidentified growth factors. Figure 4.2 shows pathways for acetate oxidation in the sulfate-reducing reactor. I have assumed that the dominant pathways for acetate oxidation are SO_4-S reduction and methanogenesis.

Assumptions about anaerobic biofilms

Several simplifying assumptions were made concerning both types of anaerobic biofilms: i) oxidation of acetate to $H_2 + HCO_3^-$ is thermodynamically favorable in a narrow range of conditions (Zehnder and Stumm 1988), I have assumed that these conditions were not present in the reactors; ii) although both NRB and SRB are capable of using H_2 as an electron donor (Tiedje 1988; Widdel 1988 respectively), I have assumed that H_2 was not available as an electron donor for reduction of NO_3-N or SO_4-S, or methanogenesis; iii) oxidation of CH_4 did not occur in either reactors; iv) S^{2-} was not oxidized by any oxidants (ie O_2, NO_3^-); v) no photosynthetic S^{2-} oxidizing anaerobes were present, vi) reduction of influent Fe^{+3} was due to bacterial respiration (Patrick and Henderson 1981; Ghiorse 1988); and vii) all C in the bacterial biomass was from acetate. Although biomass accumulation was low (less than 1.5 % of the acetate load during the high C loading rates) it was accounted for as a sink for acetate. The anaerobic respiratory pathways that are assumed to be insignificant are shown in Figures 4.1 and 4.2 as dotted lines.

Mass balance for acetate in anaerobic biofilms

Mass balances were calculated for the reactors with and without plants (controls). The terms used in the mass balance equations are presented below, units of expression are mg acetate/hour:

ACE_{IN} = Influent acetate

ACE_{OUT} = Effluent acetate

ACE_{O2} = Acetate oxidized by aerobic heterotrophs

ACE_{NO3} = Acetate oxidized by dissimilatory reduction of NO_3-N

ACE_{Fe} = Acetate oxidized by dissimilatory reduction of Fe^{+3}

ACE_{SO4} = Acetate oxidized by dissimilatory reduction of SO_4-S

ACE_{CH4} = Acetate oxidized by methanogenesis

ACE_{BIOM} = Acetate incorporated into bacterial biomass.

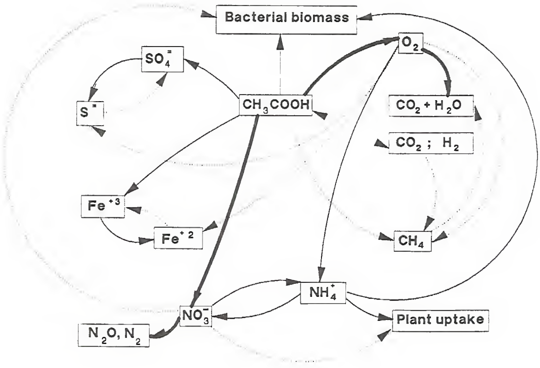


Figure 4.1. Oxidative pathways for acetate which are dominant (thick lines), accounted for (solid lines), or assumed to be insignificant (dotted lines), in an acetate mass balance in a continuous-flow stirred tank reactor with nitrate-reducing biofilms.

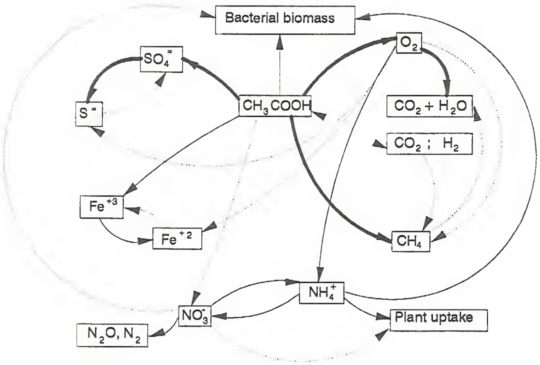


Figure 4.2. Oxidative pathways for acetate which are dominant (thick lines), accounted for (solid lines), or assumed to be insignificant (dotted lines), in an acetate mass balance in a continuous-flow stirred tank reactor with sulfate-reducing biofilm.

ACE_{CTRL} = Acetate losses in the control reactor (no plants) which are not accounted for by oxidative pathways for acetate listed above.

Equations used for mass balances in the NRB and SRB reactors without plants are:

a). Nitrate-reducing reactor

$$ACE_{IN} - ACE_{OUT} = ACE_{NO3} + ACE_{Fe} + ACE_{SO4} + ACE_{CH4} + ACE_{BIOM} + ACE_{CTRL} \qquad [4-1]$$

b). Sulfate-reducing reactor

$$ACE_{IN} - ACE_{OUT} = ACE_{Fe} + ACE_{SO4} + ACE_{CH4} + ACE_{BIOM} + ACE_{CTRL} \qquad [4-2]$$

The mass balance equation used for the NRB and SRB reactors with plants was:

$$ACE_{IN} - ACE_{OUT} = ACE_{O2} + ACE_{NO3} + ACE_{Fe} + ACE_{SO4} + ACE_{CH4} + ACE_{BIOM} + ACE_{CTRL} \qquad [4-3]$$

Equation [4-3] is the same for both types of anaerobic reactors because NH_4-N may be oxidized to NO_3-N in the aerobic microzones in rhizosphere of plants due to O_2 transport. Therefore the term for oxidation of acetate due to O_2 transport through the plants in either NRB or SRB reactors was:

$$ACE_{O2} = \qquad [4-4]$$

$$ACE_{IN} - ACE_{OUT} - ACE_{NO3} - ACE_{Fe} - ACE_{SO4} - ACE_{CH4} - ACE_{BIOM} - ACE_{CRTL}$$

Oxidation of acetate via different electron acceptors was calculated using the following stoichiometric equations:

$$CH_3COO^- + 2\ O_2 \rightleftarrows H_2O + CO_2 + HCO_3^- \qquad [4-5]$$

$$CH_3COO^- + 8/5\ NO_3^- + 8/5\ H^+ \rightleftarrows$$

$$9/5\ H_2O + CO_2 + HCO_3^- + 8/10\ N_2 \qquad [4\text{-}6]$$

$$CH_3COO^- + 8\ Fe^{+3} + 3\ H_2O \rightleftarrows$$

$$8\ Fe^{+2} + CO_2 + HCO_3^- + 8\ H^+ \qquad [4\text{-}7]$$

$$CH_3COO^- + SO_4^{=} + 1.5\ H^+ \rightleftarrows$$

$$H_2O + CO_2 + HCO_3^- + 0.5\ HS^- + 0.5\ H_2S \qquad [4\text{-}8]$$

$$CH_3COO^- + H_2O \rightleftarrows CH_4 + HCO_3^- \qquad [4\text{-}9]$$

Estimation of Oxygen Transport Based on Oxidation of NH_4-N

The fundamental assumption in this method is that NH_4-N is oxidized to NO_3^- in the rhizosphere of plants and then reduced immediately and completely to N_2 gas. The nutrient solution is continuously bubbled with N_2 therefore I have assumed that reactor water was saturated with N_2. Calculation of O_2 reduced during nitrification is based on stoichiometric oxidation of NH_4-N (WPCF 1983):

$$NH_4 + 1.83\ O_2 + 1.98\ HCO_3^- \rightleftarrows$$

$$0.021\ C_5H_7O_2N + 0.98\ NO_3^- + 1.041\ H_2O + 1.88\ H_2CO_3 \qquad [4\text{-}10]$$

Influent concentrations of NH_4-N were maintained in the nutrient solution with NH_4Cl or NH_4NO_3 salts. During the N oxidation studies these salts were replaced with $^{15}NH_4Cl$ (Cambridge Isotope Laboratories, 99 % ^{15}N) in the NRB and SRB nutrient solutions. The reactors were allowed to attain steady state concentrations of $^{15}NH_4$-N (> 4 HRTs) before $^{15}N_2$ gas samples were collected. Addition of $^{15}NH_4$-N to the reactors allowed determination of N assimilation by plants and bacteria, and nitrification.

Water Sampling

On a given day that acetate mass balance or $^{15}NH_4$-N oxidation were evaluated, reactors were sampled as follows: i) pH and Eh were measured, ii) water samples were collected, iii) influent samples and flow rates were measured, and then iv) lids were placed over the top of the reactors for collection of CH_4 or $^{15}N_2$.

Reactors were sampled intermittently for the acetate mass balance from April 30 to June 28 1992 (during PHASE II in Chapter 3) and from August 25 to Septmember 2 1992 (PHASE III in Chapter 3). The $^{15}NH_4$-N oxidation studies were done on August 30, and September 02, 1992 (during PHASE III in Chapter 3). The studies were conducted on two NRB and two SRB reactors, each set had one reactor with and one without plants. All reactors received two different acetate loading rates (Table 4.1 and 4.2). Loading rates will be referred to as low (about 9 mg/hr) and high (about 30 mg/hr) throughout the remainder of this text. The acetate mass balance was done during the high and low load. The ^{15}N oxidation study was done only during the low acetate load. The low load should provide nitrifiers with a competitive advantage for O_2 over the aerobic heterotrophs (Parker and Richards 1986; Strand 1986).

Table 4.1 Influent and effluent concentrations of major electron donors and acceptors in the nitrate-reducing reactors during two operational periods[+].

Treatment	Acetate load	n	C/N	C/S	ACE_{INF}[♥]	ACE_{EFF}	NO_3-N_{INF}	NO_3-N_{EFF}	SO_4-S_{INF}	SO_4-S_{EFF}
	mg/hr				------------------- mg/L -------------------					
No plants	30.9 (2.6)	18	1.6	20.6	630.7 (46.4)	<2.0 (na)	164.9 (29.5)	1.9 (2.7)	12.6 (1.6)	1.1 (0.9)
S.validus	31.0 (2.8)	14	1.5	22.8	636.5 (63.2)	<2.0 (na)	168.3 (20.6)	0.3 (0.4)	11.4 (1.2)	2.2 (0.5)
No plants	9.2 (0.8)	8	1.4	21.7	186.9 (13.9)	2.1 (1.4)	53.9 (2.5)	3.1 (6.7)	3.9 (2.3)	1.6 (0.7)
S.validus	9.3 (0.6)	8	1.5	23.4	191.4 (8.5)	<2.0 (na)	53.2 (3.2)	4.4 (5.3)	3.6 (2.2)	1.3 (0.5)

[+] Averages for data are presented with sample standard deviation in parentheses. Reactor pore volume = 2.2 L; Dilution rate = 0.52 day^{-1}

[♥] ACE = acetate, INF = influent, EFF = effluent

Table 4.2 Influent and effluent concentrations of major electron donors and acceptors in the sulfate-reducing reactors during two operational periods[+].

Treatment	Acetate load	n	C/S	ACE_{INF}[♥]	ACE_{EFF}	SO_4-S_{INF}	SO_4-S_{EFF}
	mg/hr			------------- mg/L ---------------			
No plants	31.0 (2.3)	18	2.2 (0.3)	619.7 (48.6)	2.7 (1.6)	116.3 (12.8)	2.1 (2.5)
S.validus	29.7 (5.0)	14	2.0 (0.4)	589.0 (97.3)	<2.0 (na)	122.9 (17.0)	2.2 (3.6)
No plants	8.9 (0.4)	8	0.8 (0.1)	181.9 (11.3)	2.5 (1.3)	98.2 (4.1)	29.8 (29.3)
S.validus	8.9 (0.5)	8	0.8 (0.1)	182.3 (11.2)	2.4 (1.5)	96.4 (4.0)	46.8 (19.5)

[+] Averages for data are presented with sample standard deviation in parentheses. Reactor pore volume = 2.2 L; Dilution rate = 0.52 day^{-1}

[♥] ACE = acetate, INF = influent, EFF = effluent

Water sampling for acetate mass balance

Redox potential and pH were measured using methods described in Chapter 3. Water samples were removed from 1-2 cm depth within the reactors. Influent samples were taken from the influent lines entering the reactor. All water samples were filtered through 0.45 μm Millipore filters and stored at 4°C in 7 ml scintillation vials. Influent flow rate was measured by collecting flow for three minutes in a 5 mL graduated cylinder.

Water samples were analyzed for acetate, SO_4-S, and NO_3-N; Total Fe, Fe^{+2}, and total soluble sulfides were analyzed one day after water sampling and gas collection (Procedures for all water chemistry are detailed in Chapter 3, Experiment II a).

Water samples for $^{15}NH_4$ oxidation and DNRA

Sampling procedures described above for the acetate mass balance were also done on days of N oxidation studies. In addition samples were analyzed for NH_4-N and NO_3-N. Forty mL of filtered samples were acidified and stored at 4°C for analysis of $^{15}NH_4$-N, and $^{15}NO_3$-N. Percent ^{15}N as water soluble NH_4-N and NO_3-N were analyzed using methods adapted from Brooks et al. (1989) and Burke et al. (1990). The technique involved volatilizing NH_4-N (with MgO), and adsorbing it on acid washed zeolite. Zeolite had been sieved through a 500 μm mesh (but retained by a 250 μm mesh), acid washed with 1N HCL, rinsed with distilled water, and dried at 80°C. About

40 mg of acid washed, dried zeolite was placed in a tin cup (Sanda Inc.) suspended from the cap of a 125 mL Nalgene polymethylpentene erlenmeyer flask. A water sample containing about 100 μg of NH_4-N, was placed in the erlenmeyer flask and diluted with 30 mL of distilled water. After dilution, 0.4 g MgO was added to the flask, which was capped immediately and swirled. The flask was then incubated for 6 days at 60°C. After incubation, the tin cups with zeolite were removed, rolled into a small ball, and analyzed for isotopic $^{15}NH_4$ by mass spectrophotometry (Hauck 1982). The sample/MgO solution was then prepared for analysis of $^{15}NO_3$-N. Fresh zeolite was placed into a new tin cup and put in the flask with the sample solution. Devardas alloy (reduces NO_3 to NH_4) was added to the solution. Flasks were capped immediately and placed in the incubator at 60°C (samples were stirred vigorously for 1 minute each day). After 6 days, zeolite was removed and analyzed for isotopic ^{15}N by isotope-mass ratio spectrophotometry.

Sampling for methane

Emission of CH_4 from NRB and SRB reactors was measured by placing Plexiglas covers over reactors (Figure 4.3) for 2 to 4 hours. A continuous flow of nutrient solution was maintained in the reactors. The sampling period was during the 12 hour light cycle (0700 to 1900). Air tight seals around the collection chamber were created by placing the base of the covers in a gutter of water (Figure 4.3). A 50 mL beaker with 20 ml of 3.7 mM zinc acetate solution was placed at the base of the plants to trap potentially toxic $H_2S_{(g)}$. Gas samples were removed through a septum in the top of the chamber using a 10 mL syringe with three-way lock-valve. Samples were injected into seven mL vacuutainers which had been evacuated and flushed (with N_2 gas) 3 to 4 times. Initially, gas sampling was done every 20 minutes for 3 hours. This intensive routine showed that CH_4 production was linear for the first 2 hours (Figure 4.4). Samples were subsequently taken every half hour for a two hour period. Methane content was analyzed within one day of sampling on a Hewlett Packard Gas Chromatograph Model 4850, using a Supelco 80/100 Poropak column. Linear regression analysis was used to determine CH_4 production rates.

Sampling for $^{15}N_2$

Emission of $^{15}N_2$ from the NRB and SRB reactors was measured by placing Plexiglas covers over the plants (Figure 4.3). A continuous flow of nutrient solution was maintained through the reactors. The cover was designed with minimum volume to concentrate $^{15}N_2$ generated in the reactors. Gas samples were removed through a septum at the top of the cover using a 10 mL syringe with three-way lock-valve. Samples were taken once every 1.5 to 2 hours during a 6 to 9 hour sampling period. The sampling period for N_2 was done during the 12 hour light cycle (0700 to 1900). Vacuutainers with samples for N_2 were sealed with silicone caulk until

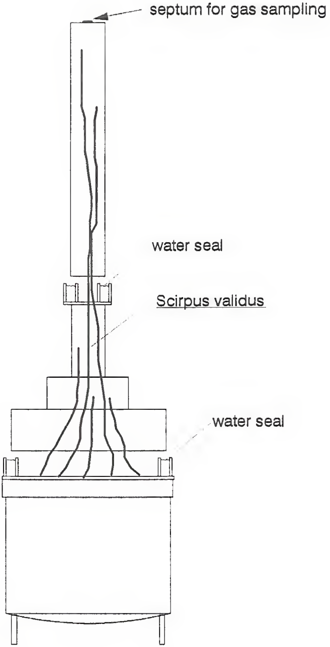
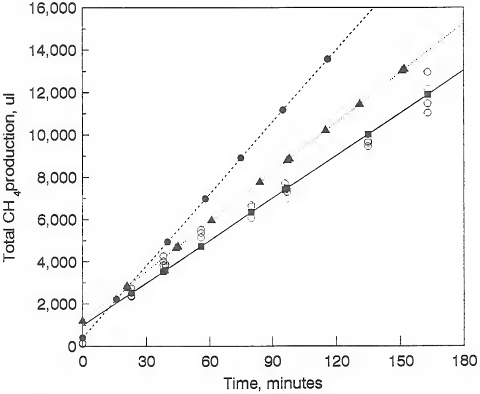


Figure 4.3. Plexiglas cover placed over plants for determining CH_4 and $^{15}N_2$ production in continuous-flow stirred tank reactors with NO_3-N and SO_4-S reducing biofilms.



Marker	Date	n	r	slope	intercept
○	9-20-91	32	0.9791	66.9	982.3
■	9-20-91	29	0.9999	66.9	982.3
▲	9-19-91	42	1.0000	78.3	1,187.0
●	8-30-91	32	1.0000	113.4	399.5

Figure 4.4. Linear production of CH_4 during a 3 hour sampling period from continuous-flow stirred tank reactors with sulfate-reducing biofilms receiving about 30 mg/hr acetate. No CH_4 was measured during an loading rate of 9 mg/hr.

they were analyzed for N_2^{15+14} and N_2^{15+15} on a mass spectrophotometer.

Dissimilatory Reduction of Nitrate to Ammonium

Nutrient solution containing $^{15}NO_3$-N (as KNO_3, 99%^{15}N) was added March 18 1992 (during PHASE IV, Chapter 3) to nutrient solution of one NRB and one SRB 4.9 L reactor. S. validus was growing in both reactors. Expected concentration entering the reactors was 12 mg/L $^{15}NO_3$-N. Water samples were analyzed for $^{15}NH_4$-N after reactors had received $^{15}NO_3$-N for about 1.3 hydraulic retention times. Reduction of NO_3-N to NH_4-N was determined by analyzing water samples for accumulation of $^{15}NH_4$-N. Samples were analyzed using methods described above for NH_4-N, NO_3-N, and $^{15}NH_4$-N. An accumulation of soluble $^{15}NH_4$-N significantly greater than background levels (0.366atom % ^{15}N) was assumed due to dissimilatory NO_3-N reduction to ammonium (Tiedje 1988).

Total Solids and Bacterial Biomass

Bacterial biomass was measured at the end of each acetate loading rate. During the low acetate loading rate bacterial biomass was removed three weeks after inital addition of $^{15}NH_4$-N to the nutrient solution. Samples were oven dried, ground in a ball mill, then analyzed for C and N on a Carla Erba C/N Analyzer, and N^{15} by mass spectrophotometry.

Plant Analysis

Five clusters of S.validus were growing in each reactor for two months under fluorescent lights (170 μeinsteins/cm^2 sec). Each cluster had 2-4 tillers per section of rhizome. Plants used during the ^{15}N studies were the same as those used during PHASEs II and PHASE III in Chapter 3. All plants were harvested after the N oxidation studies. Biomass was separated into roots and tillers (aboveground biomass), dried, ground in a Wiley mill, pulverized in a ball mill, and then analyzed for C, N and N^{15} as above.

Statistical Methods

Comparisons were made using the Statistical Analysis Systems version 6.04 (SAS 1987). A Generalized Linear model and or analysis of variance was used to compare the differences between treatments. The null hypothesis was that Observation 1 (at t_i) - Observation 2 (at t_i) = 0, i.e. there were no differences between the treatments. Significant differences are reported when $p < 0.05$.

Results

Reactor Water Chemisrty during O_2 Transport Studies

Electron donors and acceptors

Removal of acetate was greater than 98 % in the reactors receiving low and high acetate loads. Acetate concentration was less than 3 mg/L in all reactors during both loading rates (Tables 4.1 and 4.2). During the high acetate load, concentrations of electron acceptors (NO_3-N and SO_4-S) were less than 2.5 mg/L; removal was greater than 98% in the NRB and SRB reactors. The C/N ratio was similar during the low and high acetate loading rates in the NRB reactors, therefore effluent concentrations and percent removal were similar (Table 4.1). The C/S ratio was lower during the low load, therefore only 50 to 70 % of the SO_4-S was reduced in the SRB reactor.

Physico-chemical parameters: Eh and pH

Eh was lower during the high acetate loading rate than during the low loading rate in both the NRB and SRB reactors (Table 4.3). The pH was kept between 6.0 and 7.0 in all reactors. During the high load, the pH was similar with and without plants in the NRB and SRB reactors; during the low load pH was significantly lower in reactors with plants (Table 4.3).

Methane production

There was no CH_4 production measured in the NRB reactors during high or low acetate loading rates (Table 4.4). Methane production rate varied from 0.8 to 5.8 mL CH_4/hr in the SRB reactors receiving a high acetate load (Table 4.4). The rate of production was significantly greater without plants than with plants in SRB reactors. Rates of CH_4 production were negligible during the low loading rate in SRB reactors.

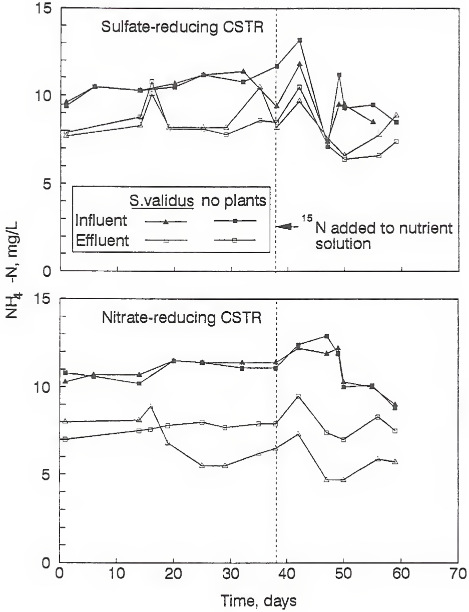


Figure 4.5. Influent and effluent NH_4-N concentrations from continuous-flow stirred tank reactors with nitrate- and sulfate-reducing biofilms receiving a low acetate loading rate. Dashed lines indicate addition of $^{15}NH_4$ (99 %).

Table 4.3 Mean Eh_7 and pH in continuous-flow stirred tank reactors (CSTR) with nitrate- and sulfate-reducing biofilms receiving low and high acetate loading rates.

Treatment	Acetate load[♥]		Sulfate-reducing CSTR		Nitrate-reducing CSTR	
		n	Eh_7	pH	Eh_7	pH
			mV		mV	
No plants	High	18	-43 (30)	6.5 (0.2)	127 (76)	6.4 (1.4)
S.validus	High	18	-15 (30)	6.6 (0.1)	98 (72)	6.0 (1.1)
No plants	Low	14	8 (17)	6.4 (0.2)	223 (21)	6.8 (0.1)
S.validus	Low	14	11 (27)	6.0 (0.5)	201 (19)	6.5 (0.1)

[+]Averages for data are presented with sample standard deviation in parentheses.

[♥]Acetate load has been rounded off to facilitate reference in the text, the actual loading rates of acetate are presented in Tables 4.1 and 4.2.

Table 4.4 Production of CH_4 and carbon (C) loads to continuous-flow stirred tank reactors (CSTR) with nitrate- and sulfate-reducing biofilm receiving low and high acetate loading rates.

			Sulfate-reducing CSTR		Nitrate-reducing CSTR	
Treatment	n	Acetate load	C load mg/L*hr	CH_4 mL/hr	C load mg/L*hr	CH_4 mL/hr
No plants	18	High	5.7 (.4)	3.8 (1.8)	5.7 (0.5)	ND
S.validus	14	High	5.5 (.9)	2.2 (1.0)	5.7 (0.5)	ND
No plants	3	Low	1.7 (.1)	ND	1.5 (0.1)	ND
S.validus	3	Low	1.6 (.2)	<0.1 (0.1)	1.6 (0.1)	ND

ND = not detected

Inorganic nitrogen

Average influent NH_4-N concentration during the low acetate load in the NRB reactors was 10.7 ± 1.4 mg/L; and 8.8 ± 1.3 mg/L in the SRB reactors. Ammonium N was significantly lower in NRB reactors with plants than without plants (Figure 4.4). There were no significant differences in NH_4-N concentrations between SRB reactors with and without plants (Figure 4.4).

Dissimilatory reduction of NO_3-N to NH_4-N. Influent and effluent concentrations of NH_4-N and NO_3-N during the DNRA study are presented in Table 4.5. Ammonium ^{15}N increased from background levels (0.366 %) to 1.2 % in the NRB and 2.3 % in the SRB reactors (Table 4.5). About 0.6 % of the influent $^{15}NO_3$-N was reduced to $^{15}NH_4$-N in the NRB reactor at an average rate of 5.1 ± 0.7 μg/hr; about 1.2 % was reduced to NH_4-N in the SRB reactor at an average rate of 10.7 ± 0.4 μg N/hr.

Estimation of Oxygen Transport Based on Acetate Oxidation

Oxygen transport was estimated by accounting for the dominant metabolic pathways involved in: i) the oxidation of acetate, and ii) the oxidation of $^{15}NH_4$ and subsequent reduction of $^{15}NO_3$-N to ^{15}N.

Percent acetate removed which was oxidized via the various respiratory pathways in the NRB and SRB reactors is shown in Tables 4.6 and 4.7.

Table 4.5 Accumulation of $^{15}NH_4$-N due to dissimilatory nitrate reduction to ammonium in continuous-flow stirred tank reactors (CSTR) with nitrate- and sulfate-reducing biofilms. The nutrient solution entering the reactors contained about 10 % Hoaglands solution, 620 ± 75 mg/L acetate, and about 13 mg/L $^{15}NO_3$-N which was 99 % ^{15}N.

Date	--- NH_4-N ---		--- NO_3-N ---		DNRA rate$^+$
Sulfate-reducing CSTR	μg/mL	% ^{15}N	μg/mL	% ^{15}N	μg/hr
March 17, 1992	8.0	0.365	13.8	0.0	na
March 19, 1992	6.6	2.358	0.03	nd	9.4
March 22, 1992	6.3	2.665	<0.02	nd	10.0
March 23, 1992	6.3	2.782	0..04	nd	10.5
averages	6.4	2.602	0.03	nd	10.0
Nitrate-reducing CSTR					
March 23, 1992 (Influent)	10.0	0.365	178	na	na
March 22, 1992	5.7	1.297	0.40	nd	4.4
March 23, 1992	7.4	1.282	0.60	nd	5.7
averages	6.5	1.290	0.50	nd	5.1

nd = not determined
na = not applicable
$^+$DNRA rate = μg/mL NH_4 * %^{15}N in NH_4 * 1/100 * Q_{inf}(mL/min) * 60

Table 4.6. Percent acetate oxidized via anaerobic respiration (NO_3-N ,Fe^{+3} and SO_4-S reduction, and methanogenesis) and/or incorporated into microbial biomass in continuous-flow stirred tank reactors with nitrate-reducing biofilms[+].

Treatment	n	Acetate load[♥]	NO_3	SO_4	CH_4	Fe^{+3}	Bacterial	Total
						%		
No plants	4	30.9 (2.6)	69.3 (7.6)	3.1 (0.2)	0.0 (0.0)	0.0 (0.1)	2.0 (na)	73.1 (8.0)
S.validus	4	31.0 (2.8)	70.6 (12.9)	3.0 (0.5)	0.0 (0.0)	0.1 (0.1)	3.1 (na)	76.8 (2.3)
No plants	11	9.2 (0.8)	56.0 (13.1)	2.3 (2.9)	0.0 (0.0)	0.1 (0.1)	8.8 (na)	68.9 (20.2)
S.validus	11	9.3 (0.6)	51.7 (7.2)	2.3 (2.5)	0.0 (0.0)	0.1 (0.1)	9.8 (na)	65.7 (13.4)

[+] Averages for data are presented with sample standard deviation in parentheses

[♥] Acetate removal was greater than 98% in during both loading rates.

Table 4.7. Percent acetate oxidized via anaerobic respiration (Fe^{+3} and SO_4-S reduction, and methanogenesis) and/or incorporated into microbial biomass in continuous-flow stirred tank reactors with sulfate-reducing biofilms[+].

Treatment	n	Acetate load[♥]	SO_4-S	CH_4	Fe^{+3}	Bacterial	Total
		mg/hr	%				
No Plants	22	31.0 (2.3)	34.1 (3.9)	32.1 (15.1)	0.1 (0.1)	2.0 (na)	68.3 (14.8)
S. validus	15	29.7 (5.0)	37.8 (5.1)	19.6 (10.1)	0.1 (0.1)	1.8 (na)	59.3 (9.7)
No Plants	11	8.9 (0.4)	82.7 (12.3)	0.0 (0.0)	0.2 (0.1)	3.6 (na)	88.0 (12.3)
S. validus	11	8.9 (0.5)	50.6 (19.1)	0.0 (0.0)	0.2 (0.1)	4.7 (na)	51.1 (18.8)

[+] Averages for data are presented with sample standard deviation in parentheses.

[♥] Acetate removal was greater than 98% in during both loading rates.

Nitrate-reducing reactors. Greater than 98% of the acetate was oxidized in all NRB and SRB reactors. However, "percent acetate accounted for" ranged from 50 to 88 % (Table 4.6). During both loading rates, there were no significant differences in "percent acetate accounted for" in reactors with and without plants.

Nitrate reduction was the predominant respiratory pathway for the oxidation of acetate (Table 4.6). There were no significant differences in NO_3^- reduction between the reactors with and without plants.

Sulfate and Fe^{+3} reduction accounted for less than 3 % of the acetate oxidized (Table 4.6). There was no methanogenesis detected in the reactors. Percent of acetate incorporated into microbial biomass was greater during the low acetate loading rate. However, rates of acetate accumulation in biomass were greater during the high load (0.58 mg/hr) than during the low load (0.37 mg/hr) in NRB reactors.

Sulfate-reducing reactors. Greater than 98% of the acetate was oxidized in both sets of reactors, however "percent acetate accounted for" ranged from 51 to 98 %. Mean values of the "percent acetate oxidized" via different respiratory pathways are presented in Table 4.7. During both the low and high loads, "percent acetate accounted for" in reactors with plants was significantly less than that accounted for in reactors without plants (Table 4.7).

Sulfate reduction in reactors with plants was significantly greater than without plants (Table 4.7). During the low acetate load, significantly less SO_4-S was reduced in reactors with plants than without plants (Table 4.7).

During high acetate load, "percent acetate oxidized via methanogenesis" in reactors with plants was significantly less than reactors without plants (Table 4.7). There was no detectable CH_4 production during the low acetate load in reactors with or without plants.

Reduction of Fe^{+3} accounted for less than 0.1% of the acetate oxidized in both reactors with and without plants (Table 4.7).

Incorporation of acetate into microbial biomass accounted for a small but significant percentage of the acetate oxidized during both low and high loads. Percent assimilated was higher during the low acetate load (Table 4.7), however mass accumulation rates were about equal during the high (0.83 mg/hr) and low loads (0.77 mg/hr).

Oxygen transport estimated with acetate mass balance. Total "percent acetate accounted for" in NRB reactors with plants were equivalent to the amount accounted for in reactors without plants. Therefore no estimates of O_2 transport through the plants based on oxidation of acetate were made for the NRB reactors.

Estimated O_2 transport through the plants in the SRB reactors during the low and high acetate loads varied from 2 to 15 g O_2/m^2 day; the averages were similar during both loading rates (Table 4.8). Estimated transport values are within ranges presented by Reed et al.(1989), and are similar to the value based on CBOD removal from field scale vegetated submerged beds in Chapter 2 (Table 4.8).

Estimation of O_2 Transport Based on Oxidation of $^{15}NH_4$ Accumulation of ^{15}N

During 8 and 10 hour periods, atom % ^{15}N steadily increased above background levels in the NRB reactor with plants (Table 4.9). No $^{15}N_2$ was detected in NRB reactors without plants, or the SRB reactors with and without plants (Table 4.9).

Estimation of O_2 transport based on N oxidation

Estimated O_2 transport through the plants in the NRB reactor was 0.8 ± 0.2 g O_2/m^2 day. There was no accumulation of $^{15}N_2$ in the SRB reactors, therefore O_2 transport was not estimated.

Mass Balance for $^{15}NH_4$ Addition

Recovery of ^{15}N added to the reactors varied from 69 to 119% (Table 4.10). Amount of ^{15}N assimilated by the tillers and roots were similar in both the NRB and SRB reactors. The majority (70 to 90%) of the ^{15}N was recovered as NH_4 in the effluent. Eighteen percent was recovered as $^{15}N_2$ above the NRB reactor with plants.

Table 4.8 Calculated O_2 transport through Scirpus validus based on acetate mass balance in continuous-flow stirred tank reactors (CSTR) with nitrate- and sulfate-reducing biofilms receiving to high and low acetate loading rates.

ACE load	Sulfate-reducing CSTR	Nitrate-reducing CSTR
mg/hr	---------- g O_2/m^2*day	------------
HIGH	9.5 ±7.7	0
LOW	10.3 ± 4.3	0
Burgoon 1992	23	na
Reed et al. 1988	5 - 45	na

$^+$surface area for growing plants was 181.1 cm ^{2} (0.0181 m^2).

Table 4.9 Increase in atom % $^{15}N_2$ due to nitrification and denitrification in two continuous-flow stirred tank reactors with nitrate- and sulfate- reducing biofilms. Reactors had no plants (NPL) or were planted with Scirpus validus (PL).

		Sulfate-reducing		Nitrate-reducing	
Date	hrs	NPL	PL	NPL	PL$^+$
		------------- atom % ^{15}N		--------------	
8/30/92	3	.3707	.3706	.3714	.3769
	6	.3707	.3706	.3723	.3877
	10	.3707	.3706	.3707	.3938
9/02/92	0	.3707	.3707	.3707	.3712
	3.5	.3705	.3705	.3714	.3772
	8	.3707	.3707	.3722	.3840

$^+$Linear regression coefficients for acccumulation of ^{15}N was 0.9718 on 8/30/92 and 0.9994 on 9/02/92.

Table 4.10 Mass balance on $^{15}NH_4$-N added to the nitrate- and sulfate-reducing continuous-flow stirred tank reactors with and without Scirpus validus.

Treatment	Inf. $^{15}NH_4$-N	Effluent $^{15}NH_4$-N		Microbe ^{15}N		Plant ^{15}N				$^{15}N_2$		Percent accounted[+]
						tillers		roots				
	µg/hr	µg/hr	%	µg/hr	%	µg/hr	%	µg/hr	%	µg/hr	%	%
Nitrate-Reducing												
No plants	498.8	345.2	69.2	na	na	na	na	na	na	0.0	0.0	69.2
S. validus	471.2	254.0	53.9	na	na	108.1	22.9	8.9	1.9	84.2	17.9	96.6
Sulfate-reducing												
No plants	393.2	329.7	83.9	0.2	.05	na	na	na	na	0.0	0.0	92.8
S. validus	400.5	364.7	91.1	0.2	.05	103.3	25.8	9.0	2.3	0.0	0.0	119.2

[+] This represents the amount of ^{15}N measured in the effluent as NH_4-N, plant and microbial N, and N_2 gas collected in a 2.85 L chamber above the plants. Natural abundance has been accounted for in all calculations. Table with values for the plants and microbial biomass are also in Appendix A-4.1. Flow rate in the 2.2 L reactors was 1.1 mL/min.

Discussion

Acetate Mass Balance to Estimate O_2 Transport

The fundamental premise in this study is that heterotrophic microbes associated with roots oxidize acetate by reducing O_2 which has diffused into the rhizosphere. Oxygen in the rhizosphere leads to competition for acetate among aerobic heterotrophs and denitrifying heterotrophs in the NRB reactor, or competition for acetate among aerobic heterotrophs, SRB, and methanogenic bacteria in the SRB reactor. Use of O_2 as an electron acceptor should create a deficit in the mass balance, i.e. the "percent acetate accounted for" in reactors with plants should be lower than in reactors without plants. This was observed in SRB reactors during both the high and low acetate loading rates (Table 4.6). An indication of competition for the electron donor is a difference in rates of SO_4 reduction or methanogenesis between reactors with and without plants. A competitive advantage is predicted by thermodynamics; SO_4 reduction is slightly more favorable than methanogenesis (25.4 versus 23.3 kJ/equiv, Zehnder and Stumm 1988). However, competition may be better evaluated using kinetic parameters (Thauer at el. 1977; Yoda et al. 1987; Rinzema and Lettinga 1989). Yoda et al. (1987) determined a half-saturation coefficient of 32.8 mg/L acetate for methanogens, and 9.5 mg/L for SRB. Acetate in all reactors was less than

5 mg/L acetate. In general, the specific growth rate and yields are higher for SRB than for methanogens (Rinzema and Lettinga 1985). The higher affinity that SRB have for acetate allow them to grow faster at lower concentrations.

Thermodynamic and kinetic analysis support the trends in the data which implied competition for the electron donor, acetate. The SRB appeared to have outcompeted the CH_4 producing bacteria for acetate during both the high and low acetate loads. During the high load, SO_4-S reduction was similar in reactors with and without plants, however methanogenesis decreased significantly in reactors with plants (Table 4.6). During the low acetate loading rates CH_4 production was insignificant in reactors with and without plants (Table 4.6). Sulfate reduction was significantly less in reactors with plants, implying that aerobic heterotrophs consumed acetate more readily than SRB, and SRB out-competed methanogen for the acetate. Therefore use of a mass balance on acetate to estimate O_2 transport is validated by the trends in data during both high and low acetate loads.

The total acetate accounted for in NRB reactors was not significantly different with or without plants. This implies that if O_2 was leaking into the rhizosphere it was not used by aerobic heterotrophs. Generally, denitrifying bacteria are facultative anaerobes with a preference for O_2 (Teidje 1988). However, research has shown that denitrification may occur in environments with low oxygen tensions (Krul 1976; Meilberg et al.1980; Robertson and Kuenen 1984). Meilberg et al. (1980) found that O_2 tension at which nitrate reductase formed was inversely proportional to growth rate. At the lowest growth rate (0.01 hr^{-1}) nitrate reductase was synthesized at a dissolved O_2 tension of 50 mm Hg, at the highest growth rate (0.15 hr^{-1}) the enzyme was formed only up to a tension of 8 mm Hg. It is possible that growth limited denitrifying bacteria out-competed the aerobic heterotrophs for acetate. If denitrifiers were not consuming the O_2 then it should have accumulated, effected the Eh, or been reduced by another redox couple. There were no signs of O_2 accumulation in the system, neither the Eh, or Fe^{+2} was significantly effected by the plants. An alternative sink for the O_2 is nitrification. Autotrophs are expected to out compete aerobic heterotrophs in C limited environments (Parker and Richards 1986: Strand 1986). This may explain the apparent inefffectiveness of the acetate mass balance in the nitrate-reducing reactor with plants.

$^{15}NH_4$ Oxidation to Estimate O_2 Transport

Research has shown that NH_4-N is oxidized to NO_3-N, and rapidly denitrified in the root zone of emergent aquatic plants (Reddy and Patrick 1986; Reddy et al. 1989). If diffusion of O_2 into the reactor is minimized, generation of $^{15}N_2$ should be a simple and more direct method for measuring

O_2 transport into the root zone than use of an acetate mass balance. The low acetate concentration in all reactors should have favored nitrifying bacteria over aerobic heterotrophs (Parker and Richards 1986; Strand 1986). the study was only done during the low acetate load, however nitrification was observed only in the NRB reactors. In the NRB reactors, an implication of the acetate mass balance and N oxidation studies was that nitrifiers out-competed the heterotrophs for O_2.

There was no measured accumulation of $^{15}N_2$ in the SRB reactors. Results from the acetate mass balance implied that aerobic heterotrophs may have out-competed nitrifiers for available O_2. The low acetate load should have created conditions that were favorable for nitrification. It is possible that sulfides, or precipitates of sulfide (FeS_2) on the roots were inhibitory to nitrifying bacteria. Nitrification had been observed in an activated sludge unit with 49 mg/L S^{2-} (Szpyrkowicz et al. 1991). Nitrification and denitrification may have been underestimated since the mass spectrometer used was not able to detect N_2O.

Critical Analysis of Methods for Estimating O_2 Transport

Reliability of mass balance

As a natural system is simulated, the bacterial diversity and complexity increase; mass balances become more difficult because there are more sinks to account for. Complete accountability of acetate in the reactors without plants should indicate that initial assumptions about the significance of the bacterial diversity and complexity was correct. However about 30% of the acetate was not accounted for during both acetate loading rates in the NRB reactor without plants. In the SRB reactor, 32% was not accounted for during the high acetate load, while only 12% was unaccounted for during the low load (Table 4.6, 4.7). The unaccounted acetate may have been due to several potential problems: i) O_2 diffusion through the low permeability Norprene tubing in recycle and influent lines, ii) unaccounted for respiratory pathways for acetate oxidation, and iii) original assumptions about anaerobic diversity were incorrect. Diffusion of air through Norprene tubing would effect the acetate mass balance by supporting microaerophilic bacteria attached to inner surfaces of the tubing. Other effects may be chemical oxidation of Fe^{+2} to Fe^{+3} which may serve and an electron acceptor for heterotrophic Fe^{+3} reducing bacteria (Ghiorse 1988) or, use of O_2 as an electron acceptor for methanotrophs or S^{2-} oxidizing bacteria. However, calculated diffusion of O_2 through the 62.5 mil thick recycle tubing predicts that less than 0.5% of the acetate should be oxidized. Light entered the reactors around the base of the plants and whenever stoppers were removed from the control for Eh, pH and water sampling. Therefore, heterotrophic photosynthetic sulfide oxidizing bacteria nay have been present (Widdel 1988, Widdel and Pfennig 1984). Sulfur, which was observed as yellow deposits in a surface film in the SRB reactors, may act an electron acceptor for dissimilation of organic C or as an electron donor for autotrophic bacteria (Gibson 1990). Other anaerobic respiratory paths which may affect the acetate mass balance are: i) Syntrophic oxidation of acetate to CO_2 and H_2 (Rinzema and Lettinga 1985; Oremland 1988; Zehnder and Stumm 1988) or, ii) formation of butyric or propionic acid from acetate, which has been observed in digested sewage sludge (Koster 1985). This amount of diversity made it impossible to account for all possible sinks for acetate.

The poor acetate mass balance during the high load in the SRB may be due to unaccounted for respiratory pathways available to methanogens. Methanogenesis was eliminated during the low acetate loading rate, probably due to the low C/S. When SRB became the dominant acetate sink the mass balance improved considerably, from 70% to 88%. However the mass balance in the NRB was 70% despite the lack of methanogenesis. Future work which requires C mass balances, should develop methods to minimize the number of anaerobic pathways.

A potential problem in reactors with plants was leakage of air around the stems during daily operations which could sustain microaerophilic bacterial communities syntrophically existing with anaerobes (Nedwell 1982; Widdel

1988; Gibson 1990) at the water surface. This could also create an unaccounted sink for acetate. To test for effects of diffusion during daily operation, the stoppers were removed from SRB reactors without plants for two weeks. No significant differences between plant and no plant reactors were observed in Eh, or CH_4 production during the two weeks. Effects of diffusion should have been equalized when CH_4 gas was collected because rubbers stoppers were removed from both reactors when Plexiglas lids were put in place for gas collection. Studies of O_2 diffusion into anaerobic muds (Teal and Kanwisher 1961; Bouldin 1968) show that up to 25% of the acetate oxidized may have been due to diffusion. Several factors may have inhibited diffusion of air into the reactors, these are i) a glossy film which covered the surface of the water in SRB reactors with and without plants, ii) low rate of diffusion of O_2 into water and, iii) continuous purging of headspace with N_2. Future work with reactors should focus on the microbiology of surface films and operational methods to minimize potential diffusive fluxes.

Exudates of organic substances from the roots were assumed to be insignificant. The lack of methanogenesis during low acetate loading rates implies that release of root exudates was minimal, or the retention time was too short for complete fermentation to acetate. Research has shown that water soluble compounds of varying molecular weights (sugars, amino acids, hormones and vitamins) are exuded from the roots of terrestrial plants, and complex carbohydrates may be released as secretions, or during cell lysis (Whipps 1990). Generation of these compounds also varies among plant types (Hedges and Messens 1990). If decomposition of released organic matter occurred it would cause an underestimation of O_2 transport due to additional release of CH_4, or acetate. I have not found any literature on the release of organic compounds from wetland plants. Research needs to be done on release of organic matter from wetland plants because it will help determine the lower limits of organic matter in the effluents from wetlands used for wastewater treatment.

Microbial biomass. When determining the acetate incorporated into bacterial biomass, no distinction was made between aerobic or anaerobic bacterial biomass. Aerobic heterotrophic microbes may use 58% of the acetate for cell growth versus 6% by methanogens (McCarty 1975). Differences between microbial solids in plants versus no plant systems were small (Table 3.12) and considered insignificant to the overall mass balance.

Precipitate in root zone. All roots in the SRB reactors (except the actively growing tips) were black with precipitated pyrite. Oxidation of Fe^{+2}, is common in the rhizosphere of aquatic plants (Green and Etherington 1977; Taylor et al. 1984) and may also have interfered with estimation of O_2 transport. Future research will use lower SO_4^{2-}-S loading rates so that less pyrite will form in the root zone.

Dissimilatory nitrate reduction to ammonium

There are several species of nitrate- and sulfate-reducing bacteria which dissimilate NO_3 to NH_4 (Teidje 1988). DNRA was measured in both the NRB and SRB reactors (Table 4.9). If NH_4 is nitrified to NO_3, but then reduced to NH_4, the O_2 used during nitrification will not be detected. Therefore, it is possible that nitrifiers were present in SRB reactors, and that O_2 transport estimates were masked because NO_3 was reduced to NH_4. Measurements in the NRB reactors may also have been affected. The DNRA studies were conducted during the high loading of acetate, however conditions during the low acetate load were also favorable for DNRA. Teidje (1988) notes that DNRA occurs in anaerobic environments which are NO_3 limited, and have at least 1 mM NH_4; nitrate was less than 0.1 mg/L, and NH_4 was about 0.4 mM in all reactors during the DNRA study. Future studies should include combined studies of $^{15}NH_4$ oxidation and DNRA for a complete N balance. The N balance was not completed in this study because the mass spectrophotometer broken down indefinitely.

Plant production

These experiments utilized plants which were not representative of natural stands. Plants were dwarfed and at much lower densities than occur in the field. The photosynthetically active radiation (PAR) was low relative to a natural ambient light (170 vs 1000 μeinsteins/cm^2 sec of PAR). Light induces convective flows of air through the plant (Dacey 1980; Armstrong et al. 1988; Grosse 1989) and would be expected to affect O_2 transport through the plants. However, Armstrong and Armstrong (1990) found that in the range of 150 to 250 μeinsteins/cm^2*sec, convective flow of gases reach their maximum in Phragmites australis. Scirpus validus may respond similarly to PAR, which may explain why estimated values of O_2 transport were similar to those found in field conditions.

Estimated Oxygen Transport

Estimated values of O_2 transport based on C and N oxidation are compared to values calculated from literature (Table 4.11). The scale of the experiments, size of plants, and technique for quantifying the effect of plants vary among the studies. Despite the variety of techniques the values all fall within similar ranges; 5 to 50 g O_2/m^2 day, based on removal of BOD and/or accumulation of O_2, and 0.5 to 5.0 g O_2/m^2 day for removal of NH_4-N. Using units of mass transport per unit volume/time, the range of values vary by two orders of magnitude; from 160 to 10800 μg O_2/L hr for removal of organic material and/or accumulation of O_2, and 30 to 19800 μg O_2/L hr for removal of NH_4-N (Table 4.11). The high values calculated from Armstrong et al. (1990) are

Table 4.11 Summary of O_2 transport estimated using C and N oxidation (and accumulation of O_2), from field and laboratory experiments comparing wastewater treatment with and without plants. O_2 transport is presented using two types of units: i) per unit area and ii) per unit pore volume.

Reference	Plant	Age	System	Oxygen mg/L	Eh mV	O_2 transport[+] $\mu g\ O_2/L*hr$	O_2 transport $g\ O_2/m^2*d$
Carpenter et al. 1983	Myriophyllum verticillatum	na	15 mL tube	1-5	> +200	700-1100	na
Dunbabin et al. 1988	Typha domingensis	5 months	10 L bucket	3-4	na	80-300	na
Moorhead and Reddy 1990	Hydrocotyl umbellata	na	635 mL flask	0.5-7	na	700 (100)	na
Lawson 1985	Phragmites communis	mature	na	na	na	na	5-45
Gersberg et al. 1986[♥]	Scirpus validus	mature	65 m^2	na	na	10800 (19000)	2.3 (4.1)
	P. australis	mature	65 m^2	na	na	4700 (16150)	1.0 (3.4)
Reddy et al. 1990	S. validus	small	500 mL flask	0.5 - 7	na	292 (35)	na
	Scirpus pungens	small	"	"	na	0 (27)	na
	Typha sp.	small	"	"	na	160 (48)	na
	Sagittaria latifolia	small	500 mL flask	"	na	160 (90)	na
Armstrong et al. 1990	P. australis	0.6 m tall	7 L	purged with N_2	na	29000	8.5
Brix 1988	P. Australis	1.4	na	na	na	0	0
Chapter 2.	S. pungens, C oxidation	mature	6 m^2	0.0	<-200	na	29
	S. pungens N oxidation	"	"	na	<-200	na	3
Chapter 4.	S. validus, C oxidation	6-12 mos	2.2 L	0	<100	3113	10
	S. validus, N oxidation	"	"	0	<200	120	0.8

[+] Values in parentheses are based on oxidation of NH_4-N,; out of parentheses are based on BOD removal and accumulation of dissolved O_2 in solution. I have assumed 4.3 g O_2/g NH_4, oxidized (WPCF 1983), and 1.5 g O_2/g BOD removed (Metcalf and Eddy 1979).

[♥] Assumed 28% porosity for gravel used by Gersberg et al. (1986), therefore pore volume equals 18.3 x 3.5 x 0.76 x 0.28 = 13.8 m^3.

based only on the accumulation of O_2 in the rhizosphere. The high rates calculated from N oxidation in Gersberg et al. (1986) may be due to the low BOD load (5.6 g/m^2 day), and mature stands of S. validus. The range of results and methods attest to the perplexity of O_2 transport through plants.

Although the calculated O_2 transport rates are similar to reported values, there are contradictions within the experiments themselves, and with the literature. Research by Mendelssohn et al. (1981) and Howes (1993) implies that oxygen tranpsort should be higher in the less reduced (nitrate-reducing) reactor. Methods used in this experiment indicated lower O_2 transport rates in the nitrate- than in sulfate-reducing reactors. Estimated O_2 transport was lower in the nitrate-reducing reactor because the acetate mass balance did not work. Estimated transport may also have been under estimated in the $^{15}NH_4$-N oxidation studies, because the mass spectrometer used, did not measure ^{15}NO or $^{15}N_2O$. There are several operational approaches which need to be implemented to improve the use these methods for estimating O_2 transport. They are: (i) lower the acetate, SO_4-S and Fe loading rates to sulfate-reducing reactors, (ii) lower the acetate and NO_3-N loading rates to the nitrate-reducing reactors, (iii) use mature plants in a greenhouse with full sunlight, and (iv) use a masss spectrometer which will detect N_2O and NO. Under these operating conditions the contradictions between methods may be elimnated; or, easier to understand, since conditions which may have negative affects on O_2 trasnport will not be present.

A common denominator in all the studies is an oxygen demand in the rhizosphere. If the oxygen demand is not great enough oxygen accumulates. The field scale results of Gersberg et al. (1986) imply that the plants do have a high capacity for O_2 transport if conditions are favorable. Armstrong et al. (1990) maintained a high demand which resulted in high transport rates. Research by Dunbabin et al. (1988) support the hypothesis that oxygen demand augments O_2 transport. Although O_2 demand did not appear to affect estimated transport rates in this study, the plants may need to be larger to see higher rates and an effect of O_2 demand. Future research on O_2 transport needs to address the question of oxygen demand, level of anaerobiosis, and bacterial communities in the rhizosphere.

Conclusions

Two methods have been presented for estimating O_2 oxygen transport through emergent aquatic plants, i) mass balance on acetate and, ii) oxidation of NH_4. Both are indirect methods, that is O_2 in solution is not measured, instead oxidation of acetate and NH_4 due to presence of oxygen are measured. The estimated O_2 transport rates are then determined from stoichiometric oxidation of C and N. The two methods were used with plants growing in continuous-flow stirred tank reactors with nitrate- and sulfate-reducing biofilms, receiving acetate loading rates of about 9 and 30 mg/hr.

Estimating O_2 transport rates using the acetate mass balance and N oxidation gave results that were similar to values estimated in other research on O_2 transport. However results from the two types of anaerobic system tested were different. In the SRB reactors, calculation of O_2 transport with acetate mass balance was supported by apparent competition for acetate amongst the SRB and methanogens. These observations were consistent during both the low and high acetate loading rates. The acetate mass balance was not useful for detecting effects of plants on acetate oxidation in the NRB reactors.

There was no oxidation of NH_4 detected in the SRB reactors. However, DNRA was measured and may have masked any nitrification in the reactor. Nitrification was observed in the NRB reactor with plants and allowed an estimated O_2 transport rate of 0.8 g/m^2 day. This rate is similar to the rate measured in a $6m^2$ constructed marsh, however it is considerably lower than rates calculated from C oxidation. The discrepancy in measurement has not been explained.

One objective of this work was to evaluate the effect of Eh on O_2 transport. However what becomes clear is that the actual aquatic chemistry and competition for electron donors and acceptors among the bacteria present in the reactors which determine the Eh, have a large impact on the measurement of O_2 transport. Therefore the research has required a reevaluation of the utility of the redox concept. In this case it is not a useful tool for understanding O_2 into the rhizosphere.

CHAPTER 5
SUMMARY AND SYNTHESIS

Summary

This dissertation examined fundamental approaches and concepts in the design of constructed wetlands for wastewater treatment. Wetland mesocosms (6 m^2 vegetated submerged beds) were set up to evaluate effects of plants and operational mode on removal of C and N from primary and secondary wastewater. Continuous-flow stirred tank reactors were then designed, built, and used in the laboratory to evaluate the oxidation of C and N in the rhizosphere of *Scirpus validus*. Rates of C and N oxidation, due to the presence of the plants, were used to infer the amount of O_2 available to bacteria in the rhizosphere. A summary of the findings, relative to original questions and objectives follows.

Field Experiments

Field experiments were designed to evaluate the role of O_2 transport through aquatic plants on wastewater treatment. A brief discussion of questions posed in the introduction follows:

(i) Do plants affect removal of C and N in vegetated submerged beds (VSBs) receiving primary and secondary effluent ?

In a mature constructed wetland plant harvesting is generally not recommended (Crites and Tchobanoglous 1992), therefore O_2 transport through plants becomes a dominant mechanism for removal of C and N. My research concurred with previous findings on the effects of plants, which has shown that plants improve C and N removal in wetlands (Gersberg et al. 1983; 1986; Wolverton et al. 1983). However, a closer look at C removal has resulted in a better understanding of C removal processes and rates in constructed wetlands. During the first 6 hours of treatment in the batch-load VSB, plants caused CBOD to be removed faster than in VSBs without plants. However, plant effects were insignificant after 18 hours. There was no explanation for the short term effect of plants on CBOD removal in VSBs. Despite >90% removal of CBOD within 24 hours (effluent CBOD <20 mg/L) during the following 48 hours, there were no significant effects of plants on NH_4-N removal. CBOD was low enough for nitrifying bacteria to outcompete heterotrophs for O_2 (Parker and Richards 1986) transported through the plants. Methane was produced throughout the 72 hour HRT in VSBs and NVSBs. Methanogenic conditions may have inhibited nitrification. After accounting for electron acceptors in the waste stream I estimated that >60% of CBOD removal was due to methanogenesis.

First-order rate coefficients calculated for BOD removal in VSBs may vary by an order of magnitude (Conley et al. 1991). This research shows that CBOD was removed rapidly in both continuous-flow and batch load VSBs receiving primary and secondary effluent. The wide range of first-order removal coefficients reported by Conley et al. (1991) was probably due to the rapid removal of CBOD; > 90% was removed within 18 hours in the VSBs. Consequently, first-order coefficients calculated from VSBs with retention times greater than one day will be smaller than coefficients calculated from VSBs with retention times less than one day. The first-order rate model was not useful for modeling CBOD removal after 24 hours.

Oxidation rate of NH_4-N in VSBs receiving secondary effluent (CBOD <20 mg/L, NH_4-N <30 mg/L) also decreased with time. Although rates in VSBs were consistently higher than nonvegetated VSBs, the rates declined during the 6 day HRT. This may have been due to the drop in water level due to evapotranspiration.

Due to problems with bed clogging at high BOD loading rates, which may be enhanced by the presence of plant roots (McIntyre and Rhia 1991), design of VSBs for treating primary sewage effluent is not recommended. However, if required, 90% CBOD removal was possible with loading rates of 37 g CBOD/m_2 day.

(ii) What are estimated O_2 transport rates through plants if calculated from changes in CBOD and NH_4-N ?

Availability of O_2 transported through plants, to bacteria in the rhizosphere, was estimated from differences in removal rates of CBOD and NH_4-N between vegetated and nonvegetated VSBs. The O_2 transport rate estimated from CBOD removal was 28.6 g O_2/m^2 day; for NH_4-N oxidation the O_2 transport rate was estimated to be 2.4 g O_2/m^2 day. The values for CBOD and N removal are within the range of values reported by Lawson (1985). The difference between estimated transport rates based on C and N oxidation has been reported in bench scale experiments (Reddy et al. 1990). The decreasing effect of plants during the hydraulic retention time has not been reported previously and at this time was unexplainable.

(iii) Does draining the VSB introduce enough O_2 to improve C and N oxidation ? Is draining more effective than plants ?

The concept of draining the VSB to pull O_2 into the gravel bed to enhance oxidation of C and N has been proposed for improving waste treatment (Seidel 1976; Brix and Schierup 1990). The affect of draining on C removal in the VSB was evaluated by comparison of first-order CBOD removal rate coefficients in batch and continuous flow VSBs. There was no significant difference between first-order rate coefficients for VSBs with HRTs of 12 and 24 hours. The significant difference between batch-load VSBs with and without plants at hydraulic retention times of 6 hours implies that plants had a greater effect on CBOD removal than draining the VSB. The insignificant effect of draining on the VSB may be due lack of O_2 storage mechanisms when the VSB was refilled. Calculations showed that the probable mechanism involved, saturation of the microbial biofilm, would store enough O_2 for removal of only about 2% of the CBOD load.

Laboratory Experiments

Laboratory experiments were designed to examine the role of O_2 transport by Scirpus validus on C and N oxidation in anaerobic wetland microcosms. The primary objective was to design a controlled Eh, pH continuous-flow stirred tank reactor which could be used to estimate the amount of O_2 available to bacteria in the rhizosphere of S. validus. The hypothesis was that O_2 transport was independent of the type of anaerobic environment in the rhizosphere. Objectives in the experiments are reiterated and discussed below:

(i) Design an anaerobic biofilm reactor with steady state Eh and pH which simulates anaerobic wetland environments, and

(ii) Establish conditions in the reactors which are conducive to growth of an emergent aquatic plant.

A continuous-flow stirred tank reactor was designed for establishment of nitrate- and sulfate-reducing biofilms on plastic media (porosity = 0.91). Steady state Eh and pH were maintained for up to 40 days in the reactors. Anaerobic conditions were maintained by purging the nutrient solution and reactor head space with pre-purified N_2. Complete-mix conditions were verified with a conservative dye tracer and depth profiles of nonconservative elements. Variations in concentrations of acetate and SO_4 were less than 1% of the influent concentrations. A recycle ratio greater than 100 was used in all reactors to insure complete mix conditions. Although the reactor was well-mixed hydraulically, the microbial biofilms and plant roots represent isolated and distinct microenvironments. Measured Eh was significantly different than predicted Eh; predicted Eh was lower than measured Eh in the SRB reactors, and higher than measured Eh in the NRB reactors.

No chemical species or reaction byproducts in the NRB reactors solution were considered toxic to aquatic plants. In the SRB reactors, Sulfate reduction produced levels of soluble H_2S which would be toxic to aquatic plants. Ferrous iron used to precipitate $S^=$, caused H_2S concentrations to be less than 5 mg/L. Ferrous iron, also a potential growth inhibitor, was kept at concentrations less than 5 mg/L. Plant growth was not optimal in the NRB and SRB reactors. Poor growth was probably due to low light levels; however, low levels of H_2S might be toxic to the freshwater plant used.

Questions posed, and answers from the research were:

(i) Is O_2 transport through plants affected by the anaerobic condition of the rhizosphere ?

Mendelssohn et al. (1981) have shown that fermentative respiration in the roots of _Spartina alterniflora_ was affected by the Eh of the rhizosphere. Howes (1993) also found that in _S_. _alterniflora_, morphological characteristics, fundamental to O_2 transport, are better developed in plants with an oxidized rhizosphere. This study did not evaluate plant morphology or fermentative status of the roots; instead, it focused on quantifying oxidation of acetate and $^{15}NH_4$-N due to plants grown in nitrate- and sulfate-reducing environments. The two methods used to estimate O_2 transport gave different results. Estimation of O_2 transport with an acetate mass balance showed no effect of plants in the NRB reactor. However the acetate mass balances in the SRB reactor showed a significant effect of the plants at acetate loading rates of 9 and 30 mg/hr. Results supported the assumption that difference in "acetate accounted for" between reactors with and without plants, was due to O_2 transport. Data showed that aerobic heterotrophs and SRB appeared to outcompete methanogens for acetate during the high acetate loading rate; during the low acetate loading rate aerobic heterotrophs apparently caused a decrease in sulfate reduction (Table 4.5). Oxidation of $^{15}NH_4$-N was observed only in the NRB reactor with plants, not in the SRB reactor. As in the field studies, O_2 transport rates estimated from C oxidation were higher than those estimated from N oxidation. Estimated O_2 transport rates from field and laboratory studies were similar in magnitude, 10 g O_2/m^2 day for C oxidation and 0.8 g O_2/m^2 day for N oxidation from the laboratory study and, 28 g O_2/m^2 day for C oxidation and 2.4 g O_2/m^2 day for N oxidation in the field study. Similar differences were observed by Reddy et al. (1990) in laboratory experiments; however, in systems with mature plants in field scale plots, O_2 transport based on N oxidation was higher than transport based on C oxidation (Gersberg et al. 1986). Further research is needed to determine if differences in C and N oxidation rate is an experimental artifact.

Net O_2 transport rates through the plants were greater in the most reduced environment. However, comparison of transport rates based on redox potential does not address specific processes involved in O_2 transport. The results may be better explained relative to bacterial ecology and competition for electron donors and acceptors. Based on studies in the SRB it appears that aerobic heterotrophs outcompeted nitrifying bacteria for O_2. However, the apparent inactivity of the nitrifiers may have been due to the high concentration of precipitated sulfide (FeS_2) in the rhizosphere of the SRB reactor. The ineffectiveness of the acetate mass balance in the NRB may be due to nitrifying bacteria outcompeting aerobic heterotrophs for O_2. However, if this were the case, N oxidation rates should have been higher than measured. Further research is required to better understand bacterial competition for electron donors and acceptors in the two types of anaerobic environments. A future study should evaluate fermentative status of the roots, arenchyma in the roots, and convective gas flows through the plants. This would provide a comprehensive use of all tools available for understanding the affect of anaerobic conditions on O_2 transport through plants.

(ii) Can O_2 transport through plants grown in anaerobic environments be accurately estimated from C and N oxidation ?

The rates estimated using these methods are in the range of data from a variety of field and laboratory experiments comparing C and N oxidation in systems with and without plants (Table 4.11). Although previous experiments have used small and large plants, and aerobic or anaerobic conditions, most O_2 transport values fall within the range presented by Lawson (1985), 5-45 g O_2/m^2 day. Further research with the reactors should focus on microbial competition and nitrification kinetics.

Synthesis

Field Studies

Field studies show that if VSBs are used for secondary treatment they may be designed to receive CBOD loads of 37 g/m^2 day with greater than 90% removal efficiency. However, at high loads the predominant removal mechanism will be methanogenic, plants will have no significant effect on treatment, and there will be negligible N removal. It is also likely that solids accumulation may clog the flow path through the gravel pores. The solids may be flushed periodically; however, it is my opinion that gravel trickling filters may be more effective for removing BOD. Trickling filters are relatively simple mechanically, require low levels of operational skill and maintenance, and require significantly less land for similar treatment goals. For example, to remove 90% of the BOD in a VSB receiving 3800 m^3/day (1 mgd) of primary wastewater (influent BOD = 200 mg/L, k_{20}= 3.0 days^{-1}, gravel porosity = 0.28, d = 0.4 m), 2.6 hectares of land and 10400 m^3 of gravel would be required. Treating a similar waste-stream in a gravel trickling filter (2.4 m deep) and obtaining > 85% BOD removal and almost complete nitrification only requires 0.2 hectares of land, and 4900 m^3 of gravel would be required (Hall 1937, Metcalf and Eddy 1979). An additional advantage of trickling filters is that they have been used for 50 years, so the operational problems with Psychoda flies, odors and suspended solids are well understood. Use of VSBs is limited and currently have experienced problems with clogging, and limited nitrification. Perceived advantages of VSBs are no odor or insect problems, and that they are available for public access (Reed and Brown 1992). Attempts to improve nitrification in VSBs (Reed and Brown 1992) will increase operational requirements and costs. VSBs may be most practical for treating waste flows from small communities and household units. Therefore, comparisons based on 3800 m^3/day may be impractical since 90% of the VSBs in operation in the United States treat less than 3800 m^3/day; the median flow is 210 m^3/day (Reed and Brown 1992). For the above treatment problem, only 0.65 ha of land would be required to treat the flow for 250 people (95 m^3/day). Remote rural communities generally need treatment systems that are effective, have low power requirements, require unskilled maintenance, are attractive in appearance, and are not environmental nuisances (Lowe 1990). Although 4x the land and gravel required for BOD removal would be required to obtain N concentrations < 5 mg/L (Gersberg et al. 1986), the above effluent criteria could be met using VSB with an additional benefit of wildlife enhancement. Units have been installed in parks with public access (Reed and Brown 1992) and homes to replace septic tank drain fields (Fetter et al.

1976, Wolverton 1984). These are particularly appropriate in areas with high water tables or bedrock with no soil development. There is also potential for alleviating the costs incurred by municipalities to incorporate new housing developments, trailer and/or industrial parks into the waste treatment networks. Designing constructed wetlands for onsite treatment would eliminate expensive sewerage systems and plant expansions.

Laboratory Studies

Reed and Brown (1992) note that it is essential for the future of VSB technology that the problem of O_2 availability in the rhizosphere of the wetland plants be solved. Laboratory research was conducted to help understand and quantify O_2 transport through plants, and availability of O_2 to bacteria in the rhizosphere of wetland plants.

Reactor design

Theoretically, this reactor design is more realistic for simulating anaerobic wetland environments than the currently used design (Reddy et al. 1976). Two fundamental differences between the two approaches are: i) my design uses electron acceptors available in anaerobic environments to control Eh instead of pulsing O_2 into the reactor, and ii) an inert plastic substrate (used as a rooting matrix and surface for establishment of anaerobic biofilms) is used in instead of a soil. This research, along with that of many other has shown that predicted (using the Nernst equation) and measured Ehs differ significantly. Therefore Eh is not useful for determining precise information about reactor chemistry. Establishing anaerobic microcosms may provide a better environment for studying wetland biogeochemistry than systems with "controlled" Eh. The reactors were designed for conducting C mass balances; therefore, soil was not used because of interference from soil organic matter. It may be possible to design reactors which use soils so specific soil properties may be accounted in biogeochemistry research. The effects of microenvironments in the plant roots and in biofilms need to be examined further.

Oxygen transport

Plants have beeen shown to have an effect on the Eh (Armstrong 1967; Armstrong et al. 1990; Carpenter et al. 1983; Mendelssohn et al. 1981; Dunbabin et al. 1988). These observed effects were probably due to experimental design. Most experiments placed plants in small containers of solution with little or no O_2 demand. Therefore O_2 accumulated rapidly and an Eh effect was recorded (Armstrong et al. 1990; Carpenter et l. 1983). My experiments established plants in steady state environments with O_2 demand greater than the O_2 transport rate through the plants, therefore O_2 did not accumulate and no significant effects of Eh were observed.

Results were explained in terms of bacterial competition for electron donor and acceptors. Oxidation-reduction-potential was useful in showing that no oxygen accumulated in the reactors. However, bacterial processes involved with O_2 transport may be better addressed with questions such as: "Is O_2 transport affected by the O_2 demand in the rhizosphere ?" and/or "How does bacterial competition for O_2 affect oxidation of C and N in anaerobic environments ? In this research, O_2 transport in the SRB appeared to be independent of the acetate load (load can be considered to be analogous to O_2 demand). Research by Dunbabin et al. (1988) has shown that O_2 availability in the rhizosphere may be related to O_2 demand. The O_2 demand in the rhizosphere may effect O_2 stress on plants and result in the small and large growth forms observed by Mendelssohn et al. (1981) and Howes (1993). Further research in these reactors with mature plants and varying O_2 demand may clarify possible effects of O_2 demand in the rhizosphere.

Armstrong et al. (1990) have designed a system which constantly flushes deoxygenated water past the roots to maintain a high "soil oxygen demand". The method used by Armstrong et al. (1990) makes direct measurements of O_2; therefore, it does not provide insights into microbial competition for O_2 in the rhizosphere. However, the bacterial diversity in the reactors result in numerous assumptions about the complexity of anaerobic ecosystems.

An accurate materials balance becomes difficult due to the numerous pathways possible in the ecosystem. The complexity of the methanogenic consortia seemed to have been eliminated by supplying excess SO_4 in the SRB reactor. Although the bacterial complexity of the nitrate-reducing reactor should have been less, the mass balance still only accounted for about 70% of the acetate. Potential leaks of O_2 into the reactors, and control of bacterial diversity need improvement; otherwise complete mass balances are lost in a complicated web of biological diversity.

The loading rates of acetate, NO_3-N, and SO_4-S used in the reactors were higher than most natural systems. The SO_4-S reduction rates were high; higher than SO_4 reduction rates in both acid mine drainage wetlands and estuaries. Effects of plants in the rhizosphere such as exudation of organic matter, acidification from oxidation of NH_4-N, and removal of nutrients may also have greater impact on reactor chemistry at lower loading rates. Measuring levels of ADH in the roots may provide new insights in to how plants adapt to varying O_2 demand in the rhizosphere.

Application of reactors for environmental research

Nurseries for wetland plants are growing in number as wetland construction for mitigation, waste treatment, and restoration expands. It may be useful to screen species for O_2 transport capacities, nutrient assimilation, and resilience under different environmental stresses. Research is beginning to focus on nutrient requirements of wetland plants in anaerobic environments. However researchers are working with reductants not found in wetlands, such as titanium citrate, in attempts to create anaerobic environments for growing the plants (Brix, personal communication). My reactors allow creation of nitrate- and sulfate-reducing, and methanogenic environments for studying nutrient requirements of plants. This reactor design has potential use for studies on larger, mature plants. This will require new research efforts because the larger root mats in mature plants may create larger anaerobic microzones and have different effects on rhizosphere chemistry.

Other areas of research in which reactors may be used are: i) effect of rhizosphere toxins such as H_2S, and Fe^{+2} on plant growth, ii) degradation of pesticides in anaerobic wetland environments, iii) removal rates and fate of heavy metals (Pd, Cd, Zn, As, Se) in wetlands, and iv) nutrient cycling and microbial competition.

APPENDIX
SUMMARY DATA TABLES

Table A-2.1. Data used to calculate a CBOD/BOD ratios in the conversion of BOD measurements to CBOD in Table 3.

Date	Tank	BOD	CBOD	CBOD/BOD Mean	CBOD/BOD CV
		---- mg/L ----			%
S.latifolia					
6-29-90	3	16.9	5.6	0.374	28.3
	6	10.2	5.6		
7-06-90	3	24.4	6.2		
	6	21.6	6.3		
7-09-90	3	11.4	4.7		
	6	11.6	4.7		
S.pungens					
6-29-90	4	16.1	8.4	0.333	29.4
7-06-90	4	33.3	10.4		
7-09-90	4	22.2	5.5		
H. umbellata					
6-29-90	1	51.7	25.9	0.624	21.8
	2	43.6	26.3		
7-06-90	1	58.3	45.4		
	2	57.8	26.5		
7-09-90	1	16.1	12.1		
	2	24.4	15.1		

Table A-2.2 First-order CBOD removal rate coefficients for batch and continuous flow tanks. Coefficients are calculated for the actual tank temperature.

Treatment	HRT (days)			
	0.25 n	0.5 n	0.75 n	1.0 n
	days-1			
BATCH				
S. pungens	6.5 ± 1.3 (9)	4.2 ± 0.2 (9)	3.0 ± 0.1 (9)	2.3 ± 0.2 (9)
No plants	3.5 ± 2.0 (9)	4.2 ± 0.4 (9)	4.0 ± 0.5 (9)	3.3 ± 0.6 (9)
CONTINUOUS				
S. pungens	NA	6.7 ± 0.9 (24)	NA	2.7 ± 0.2 (6)

Table A-3.1. Preparation of stock solutions of salts for addition to 8 liter nutrient cisterns.

Salt	Stock Solution	Sulfate Reduction	Nitrate Reduction
	--- g/L ---	-------- mL/8L ---------	
$NaC_2H_4O_2$	320.0	40	40
K_2SO_4	79.2	60/32	0
NH_4SO_4	3.6	60/32	0
$FeSO_47H_2O$	172.6	25	1/0.5
K_2HPO_4	50.6	25	25
$CaCl_22H_2O$	66.1	25	25
$MgCl_26H_2O$	50.2	25	25
$MnCl_24H_2O$	1.4	25	25
$ZnCl_2$	0.8	25	25
KI	0.7	25	25
NH_4Cl	11.4	25	25
KNO_3	200.0	0	50
NH_4NO_3	17.2	0	25
Yeast Extract	2.4	10	0

Table A-4.1 Final plant weights and bacterial biomass accumulation rates used in $^{15}NN_4$-N mass balance in the nitrate- and sulfate-reducing continuous flow stirred tank reactors.

	Tillers	Roots	no plants	*S. validus*
	- gs (dry) -		------- mg/hr ------	
Nitrate-reducing CSTR	30.9	2.7	0.3083	0.4083
Sulfate-reducing CSTR	49.7	2.6	0.8042	0.8917

APPENDIX 4.2

Basic conversion ratios used for estimating oxidation of acetate dueue to oxygen transport through plants. Conversion ratios used for calculating oxidation of acetate for the measured removal of electron acceptors. All stoichiometric equations are from McCarty (1976).

Stoichimetric mass balances

a. Oxygen as an electron acceptor

$CH_3COO^- + 2O_2 \quad CO_2 + HCO_3^- + H_2O$

1.0847 mg O_2/mg acetate not accounted for

b. NO_3 as an electron acceptor

$8/5\ CH_3COO^- + NO_3^- + H^+ \quad 5/8\ HCO_3^- + 9/8\ H_2O\ 5/8\ CO_2 +$
$1/2N_2$

2.6339 mg acetate/mg NO_3-N removed

c. Fe^{+3} as an electron acceptor

$CH_3COO^- + 8Fe^{+3} + 3H_2O \quad 8Fe^{+2} + CO_2 + HCO_3 + 8H^+$

0.1321 mg acetate/mg Fe^{+3} removed

d. SO_4 as an electron acceptor

$CH_3COO^- + SO_4 + 3/2H^+ \quad 1/2\ HS^- + 1/2\ H_2S + HCO_3 + CO_2$

1.8438 mg acetate/mg SO_4-S removed

e. Acetate as an electron acceptor, methanogenesis.

$CH_3COO^- + H_2O \quad CH_4 + 1/8\ HCO_3^-$

2.6339 mg acetate/mL CH_4 produced

f. Biomass production

Biomass = $C_5H_{12}N_2O_3$ Molecular weight = 148

Biomass accumulation rate from Table 3.12 used for each reactor (BIO, mg/day).

BIO * 60 C/148 * 59 ace/24 C *day/24 hr = acetate into biomass, mg/hr

APPENDIX 4.3

Procedure used for calculating oxigen transport based on oxidation of NH_4-N.

Basic approach involved calculations of: 1. total N_2 initially in the gas collection chamber, 2. accumulation of N due to reduction of influent NO_3-N, 3. amount of ^{15}N accumulating in gas collection chamber due to oxidation and subsequent reduction of $^{15}NH_4$, 4. linear regression of accumulation of ^{15}N versus time.

Calculations:

1. Total N_2 initially in gas collection chamber

78.084% N_2 * 1/100 * 2850 mL * 1 mmole/22.4 L

2. Accumulation of N_2 due to reduction of influent NO_3

0.0785 mmoles N/hr * time * 1/2

3. ^{15}N accumulation from $^{15}NH_4$ oxidation

[(atom % ^{15}N measured - 0.3707)/99] * (Total N_2 + N_2 from NO_3)

4. Enrichment rate

linear regression: time of sampling (t_i) vs. mmoles ^{15}N (t^i)

mmoles ^{15}N/hr * 15 mg/mmole = mg N oxidaized/hr

5. Oxygen transport rate

4.3 mg O_2/mg N oxidized * mg N oxidized/hr = mg O_2/hr

REFERENCES LIST

Allam, A. I. and Hollis, J. P. 1972. Sulfide Inhibition of oxidases in rice roots. Phytopathology. 62:634-639.

Almgren, T., and Hagstrom, I. 1974. The oxidation rate of sulfide in seawater. Water Res. 8:395-400.

APHA. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. American Public Health Association, Washington, DC.

Armstrong, J., and Armstrong, W. 1988. Phragmites australis a preliminary study of soil-oxidizing sites and internal gas transport pathways. New Phytologist. 108:372-382.

Armstrong, J., Armstrong, W., and Beckett, P. M. 1988. Phragmites australis: A critical appraisal of the ventilating-pressure concept and an analysis of resistance to pressurized gas-flow and gaseous diffusion in horizontal rhizomes. New Phytologist. 110:383-389.

Armstrong, J. and Armstrong, W. 1990a. Pathways and Mechanisms of oxygen transport in Phragmites australis. pp 529-533. In Cooper, P. F. and Findlater, B. C. (ed), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Armstrong, J., and Armstrong, W., 1990b. Light-enhanced convective through flow increases oxygenation in rhizomes and rhizosphere of Phragmites australis (Cav.) Trin. ex steud. New Phytologist. 114:123-128.

Armstrong, W. 1967. The oxidizing activity of roots in waterlogged soils. Physiologica Plantarum. 20: 920-926.

Armstrong, W., Armstrong, J., and Beckett, P. M. 1990. Measurement and Modeling of oxygen release from roots of Phragmites australis. pp 41-51. In: Cooper, P. F. and Findlater, B. C. (ed), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Austin, B. (ed). 1988. Methods in Aquatic Bacteriology. Wiley Interscience. New York.

Bedford, B. L., Bouldin, D. R., and Beliveau, B. D. 1990. Net oxygen and carbon dioxide balances in solutions bathing roots of wetland plants. Unpublished manuscript.

Benefield, L. A., Judkins, J. F., and Weand, B. L. 1982. Process Chemistry for Water and Wastewater Treatment. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Bouldin, D. R. 1968. Models for describing the diffusion of oxyen and other mobile constituents across the mud-water interface. J. Ecol. 56:77-87.

Brix, H. 1990. Gas exchange through the soil-atmosphere interphase and through dead culms of Phragmites australis in constructed reed bed receiving domestic sewage. Water Res. 24:259-266.

Brix, H., and Schierup, H. H. 1990. Soil oxygenation in constructed reed beds: the role of macrophyte and soil-atmosphere interface oxygen transport. pp 53-66. In: Cooper, P. F. and Findlater, B. C. (ed), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Brooks, P.D., Stark, J. M., McInteer, B. B., and Preston, T. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Science Society of America Journal. 53:1707-1711.

Bucksteeg, K. 1987. Sewage treatment in helophyte beds-First experiences with a new treatment procedure. Wat. Sci. Tech. 19(10):1-10.

Burgoon, P. S. 1989. Wastewater treatment in vegetated submerged beds with artificial substrates. Master of Engineering. University of Florida, Gainesville.

Burgoon, P. S., Reddy, K. R., and Koopman, B. 1991. Vegetated submerged beds with artificial substrates. I: BOD removal. J. Envir. Engrg. ASCE. 117(4):394-407.

Burke, I. C., Masier, A. R., Porter, L. K., and O'Deen, L. A. 1990. Diffusion of soil extracts for nitrogen and nitrogen-15 analyses by automated combustion/Mass spectrometry. Soil Science Society of America Journal. 54:1190-1192.

Carpenter, S. R., Elser, J. J., and Olson, K. M., 1983. effects of roots of Myriophyllum verticillatum L. on sediment redox potential. Aquat. Bot. 17:243-249.

Characklis, W. G., and Marshall, K. C. 1990. Biofilms. John Wiley and Sons, New York.

Chen, K. Y., Asce, A. M., and Morris, J. C. 1972. Oxidation of sulfide by O_2: catalysis and inhibition. J. Sanit. Eng. Div. Proc. Am. Soc. Civ. Eng. 98:215-227.

Cleemput, O. V., Patrick, W. H., and McIlhenny, R. C. 1975. Formation of chemical and biological denitrification products in flooded soil at controlled pH and redox potential. Soil Biol. Biochem. 7:329-332.

Cline, J. D., and Richards, F. A. 1969. Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature and pH. Environ. Sci. Technol. 3:838-843.

Conley, L. M., Dick, R. I., and Lion, L.W. 1991. An assessment of the root zone method of wastewater treatment. J. Water Pollut. Control Fed. 63:239-247.

Connell, W. E., and Patrick, W. H. 1968. Sulfate reduction in soil: Effects of redox potential and pH. Science. 159:86-97.

Cooper, P. F. 1990. European Design and Operations Guidelines for Reed Bed Treatment Systems. Prepared for the EC/EWPCA Emergent Hydrophyte Treatment Systems Expert Control Group, Water Research Centre. Swindon, Wiltshire, United Kingdom.

Cooper, P. F., and Findlater, B. C. (eds) 1990. Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Crites, R. W., and Tchobanoglous, G. 1992. Discussion of: Nitrogen removal in experimental wetland treatment systems: Evidence for the role of aquatic plants. Water Environment Research. 64(7):920.

Dacey, J.W. H. 1980. Internal winds in the water lilies: An adaptation to life in the anaerobic sediments. Science. 210:1017-1019.

Dacey, J. W. H. 1981. Pressurized ventilation in the yellow water-lily. Ecology. 62:1137:1147.

DeBusk, T. A., Langston, M. A., and Burgoon, P. S. 1990. A performance comparison of vegetated submerged beds and floating macrophytes for domestic wastewater treatment. pp 301-308. In: Cooper, P. F. and Findlater, B. C. (eds), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Delaune, R. D., Smith, C. J., and Tolley, M. D. 1984. The effect of sediment redox potential on nitrogen uptake, anaerobic root respiration, and growth of Spartina alterniflora Loisel. Aquatic Botany. 18:223-230.

Dunbabin, J. S., Pokorny, J., and Bowmer, K. H. 1988. Rhizosphere oxidation by Typha domingensis, pers., in miniature artificial wetland filters used for metal removal from wastewaters. Aquatic Botany. 29:303-317.

Eckert, W. 1991. Simulation of metalimnetic redox conditions in a chemostat system. Israel Oceanographic and Limnological Research Biennial Report, 1990-1991, Haifa, Israel.

Fetter, C. W., Sloey, W. E., and Spangler, F. L. 1976. Potential replacement of septic tank drain fields by artificial marsh wastewater systems. Groundwater. 14:396-402.

Gale, P. M., Devai, I., Reddy, K. R., and Graetz, D. A. 1993. Denitrification potential of soils in constructed and natural wetlands. In review.

Gearheart, R. A., Klopp, F., and Allen, G. 1989. Constructed free surface wetlands to treat and receive wastewater: Pilot project to full scale. pp 121-135. In: Hammer, D. A. (ed), Constructed Wetlands for Wastewater Treatment: Municipal, Industrial, and Agricultural. Lewis Publishers Inc., Chelsea, Michigan.

Gersberg, R. M., Elkins, B. V., and Goldman, C. R. 1983. Nitrogen removal in artificial wetlands. Water Res. 17:1009-1014.

Gersberg, R. M., Elkins, B. V., Lyon, S. R., and Goldman, C. R. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. 20:363-368.

Ghiorse, W. C. 1988. Microbial reduction of manganese and iron. pp 305-353. In: Zehnder, A.J.B. (ed), Biology of Anaerobic Microorganisms. John Wiley and Sons, New York.

Gibson, G. R. 1990. A review: Physiology and ecology of the sulphate-reducing bacteria. J. Appl. Bacteriol. 69:769-797.

Green, M. S. and Etherington, J. R. 1977. Oxidation of ferrous iron by rice roots: A mechanism for waterlogging tolerance. Journal of Experimental Botany. 20:678-690.

Grosse, W., and Mevi-Schutz, J. 1987. A beneficial gas transport system in Nymphoides peltata. American Journal of Botany. 74:941-952.

Grosse, W. 1989. Thermo-osmotic air transport in aquatic plants affecting growth activities and oxygen diffusion to wetland soils. pp 469-476. In: Hammer, D. A. (ed), Constructed Wetlands for Wastewater Treatment: Municipal, Industrial, and Agricultural. Lewis Publishers, Inc., Chelsea, Michigan.

Hammer, D. 1989. Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural. Lewis Publishers, Chelsea, Michigan.

Hall, G. A. 1937, Trickling filter loadings. Sewage Works Journal. 9:50-62.

Hauck, R. D. 1982. Nitrogen-isotope-ratio analysis. pp 735-776. In: Page, A. L., Miller, R. H., and Keeney, D. R. (eds), Methods of soil Analysis. Part 2 Chemical and Microbiological Properties (2nd ed.), American Society of Agronomy Inc., Madison, Wisconsin.

Hedges, R. W., and Messens, E. 1990. Genetic aspects of rhizosphere interactions. pp 129-176. In: Lynch, J. M. (ed). The Rhizosphere. John Wiley and Sons, New York.

Herlihy, A. T., and Mills, A. L. 1985. Sulfate reduction in freshwater sediments receiving acid mine drainage. Applied and Environmental Microbiology. 49:179-186.

Hillel, D. 1982. Introduction to Soil Physics. Academic Press, Inc., London, England

Holm, T. R., and Curtiss, C. D. 1989. A comparison of oxidation-reduction potentials calculated from the As(V)/As(III) and Fe(III)/Fe(II) couples with measured platinum-electrode potentials in groundwater. J. Cont. Hydrol. 5:67-81.

Howeler, R. H. 1973. Iron induced oranging disease of rice in relation to physico-chemical changes in a flooded oxisol. Soil Sci. Soc. Amer. Proc. 37:898-903.

Howes, B. L., Dacey, J. W. H., and King, G. M. 1984. Carbon flow through oxygen and sulfate reduction pathways in salt marsh sediments. Limnol. Oceanogr. 29:1037-1051.

Howes, B. L., Howarth, R. W., Teal, J. W., and Valiela, I. 1981. Oxidation-reduction potentials in a salt marsh: Spatial patterns and interactions with primary production. Limnol. and Oceanogr. 26:350-360.

Howes, B. L. 1993. Internal oxygen transport by Spartina alterniflora: Relationship to rhizosphere oxidation and growth form. Presented at the Second Biennial Symposium on Biogeochemisrty of Wetlands. Baton Rouge, Louisiana.

IAWQ. 1992. Wetlands Downunder: Wetland Systems in Water Pollution Control. Proceedings of an International Specialist Conference. University of New South Wales, Sydney, Australia.

Jackson, J. A. 1990. Man-made wetlands for wastewater treatment: Two case studies. pp 574-580. In: Hammer, D. A. (ed), Constructed Wetlands for Wastewater Treatment: Municipal, Industrial, and Agricultural. Lewis Publishers Inc., Chelsea, Michigan.

Jackson, R. E., and Patterson, R. J. 1982. Interpretation of pH and Eh trends in a fluvial-sand aquifer system. Water Resour. Res. 18:1255-1268.

JCPDS. 1980. Mineral Powder Diffraction File. International Center for Diffraction Data. Swarthmore, PA.

Kleinmann, R. L. P., and Girts, M. A. 1987. Acid mine water treatment in wetlands: An overview of an emergent technology. pp 255-262. In: Reddy, K. R. and Smith, W. H. (eds), Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing Inc., Orlando, FL.

Koch, M. S., Mendelssohn, I. A., and Mckee, K. L. 1990. Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol. Oceangr. 35:399-408.

Koster, I. W. 1985. Microbial, chemical, and techonological aspects of the anaerobic digestion of organic pollutant. pp 286-311. In: Wise, D. L. (ed). Biotreatment Systems; Volume I. CRC Press Inc., Boca Raton, FL.

Kristjansson, J. K., Schoneit, P., and Thauer, R. K. 1982. Different K_s values for hydrogen of methanogenic bacteria and sulfate-reducing bacteria: An explanation of the apparent inhibition of methanogenesis by sulfate. Arch. Microbiol. 131:278-282.

Krul, J. M., and Veeningen, R. 1977. The synthesis of the dissimilatory nitrate reductase under aerobic conditions in a number of denitrifying bacteria isolated from activate-sludge and drinking water. Water Res. 11:39-43

Lawrence, A. W., and McCarty, P. L. 1966. The effects of sulfides on anaerobic treatment. Air and Water Pollut. Int. J. 10:207-221.

Lovely, D. R., and Klug, M. J. 1986. Model for the distribution of sulfat.e reduction and methanogenesis in freshwater sediments. Geochemica et Cosmochimica Acta. 50:11-18.

Lowe, N. R. 1990. The need for hydrophyte-based systems in the treatment of wastewater from small communities. pp 1-9. In: Cooper, P. F. and Findlater, B. C. (ed), Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford, England.

Lowe, E. F., Stites, D. L., and Bartoe, L. E. 1989. Potential role of marsh creation in restoration of hypertrophic lakes. pp 710-717. In: Hammer, D. (ed), Constructed Wetlands for Wastewater Treatment: Municipal, Industrial, and Agricultural. Lewis Publishers Inc., Chelsea, Michigan.

Masscheleyn, P. H., Delaune, R. D., and Patrick, W. H. 1990. Transformations of selenium as affected by sediment oxidation-reduction potential and pH. Env. Sci. and Tech. 24:91-97.

Masscheleyn, P. H., Delaune, R. D., and Patrick, W. H. 1991. Arsenic and selenium chemistry as affected by sediment redox potential and pH. J. Environ. Qual. 20:522-527.

McCarty, P. L. 1975. Stoichiometry of biological reactions. Progress in Water Technology. 7(1):157-172.

McCarty, P. L., Beck, L., and St. Amant, P. 1969. Biological denitrification of wastewater by addition of organic materials. Proceedings of the 24th Industrial Wastes Conference. Purdue Univ., Lafayette, Indiana.

McIntyre, B. D., and Rhia, S. J. 1991. Hydraulic conductivity and nitrogen removal in an artificial wetland system. J. Environ. Qual. 20:259-263.

McKee, K. L., Mendelssohn, I. A., and Burdick, D. M. 1989. Effect of long-term flooding on root metabolic response in five freshwater marsh plant species. Can. J. Bot. 67:3446-3452.

Meilberg, J. B. M., Bruinenberg, P. M., and Harder, W. 1980. Effect of dissolved oxygen tension on the metabolism of methylated amines in Hypomicrobium X in the absence and presence of nitrate: Evidence for "aerobic" denitrification. J. Gen. Microbiol. 120:1645-1650.

Mendelssohn, I. A., McKee, K. L., and Patrick, W. H. 1981. Oxygen deficiency in Spartina alterniflora: Metabolic adaptation in anoxia. Science. 214:439-441.

Metcalf and Eddy, Inc. 1979. Wastewater engineering: Treatment Disposal Reuse. McGraw-Hill Book Company. New York.

Michelson, A. R., Jacobson, M. E. Scranton, M. I., and Mackin, J. E. 1989. Modeling the distribution of acetate in anoxic estuarine sediments. Limnol. Oceanogr. 34:747-757.

Middlebrooks, E. J., Middlebrooks, C. H., Reynolds, J. H., Watters, G. Z., Reed, S. C., and George, D. B. 1982. Wastewater Stabilization Lagoon Design, Performance, and Upgrading. Macmillan Publishing Co. Inc., New York.

Monteith, H. D., Bridle, T. R., and Sutton, P. M. 1980. Industrial waste carbon sources for biological denitrification. Progress in Water Technology, 12:127-141.

Moorhead, K., and Reddy, K. R. 1988. Oxygen transport through selected aquatic macrophytes. J. Environ. Qual. 17:138-142.

Moorhead, K. K. and Reddy, K. R. 1990. Carbon and nitrogen transformations in wastewater during treatment with Hydrocotyl umbellata L. Aquatic Botany. 37:153:161.

Moraghan, J. T., and Patrick, W. H. 1974. Selected metabolic processes in a submerged soil at controlled pH values. Transactions of the 10th International Congress of Soil Science. 2:264-269.

Nedwell, D. B. 1982. The cycling of suphur in marine and freshwater sediments. pp 73-106. In: Nedwell, D. B. (ed). Sediment Microbiology. London, Academic Press.

Olila, O. 1992. Reactivity of inorganic phosphorus in sediments of shallow eutrophic lakes. Ph.D, University of Florida, Gainesville.

Oremland, R. S. 1988. Biogeochemistry of Methanogenic Bacteria. pp 641-706. In: Zehnder, A. J. B. (ed), Biology of Anaerobic Microorganisms. John Wiley and Sons, New York.

Parker, D. S., and Richards, T. 1986. Nitrification in trickling filters. J. Water Pollut. Contr. Fed. 58:896-902.

Patrick, W. H. 1966. Apparatus for controlling the oxidation-reduction potential of waterlogged soils. Nature. 212:1278-1279.

Patrick, W. H. 1981. The role of inorganic redox systems in controlling reduction in paddy soils. p.107-117. In: Institute of Soil Science (ed), Proceedings of Symposium on Paddy Soil. Science Press, Springer-Verlag, Beijing, China.

Patrick, W. H., Jr., Williams, B. G., and Moraghan, J. T. 1973. A simple system for controlling redox potential and pH in soil suspensions. Soil Science Society of America Proceedings. 37:331-332.

Pedrazzini, F. R., and McKee, K. L. 1984. Effect of flooding on activities of soil dehydrogenases and alcohol dehydrogenase in rice (Oryza sativa) roots. Soil Sci. Plant. Nutr. 30:359-366.

Pfennig, N. 1989. Metabolic diversity among the dissimilatory sulfate-reducing bacteria. Antoine Leeuwenhoek Micriobiol. 56:127-138.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

Reddy, C. N., Jugujinda, A., and Patrick, W. H. 1976. Systems for growing plants under controlled redox potential-pH conditions. Agron. J. 68:987-989.

Reddy, K. R., and Patrick, W. H. 1986. Denitrification losses in flooded rice fields. Fertilizer Research. 9:99-116.

Reddy, K. R., and Smith, W. H. (eds), 1988. Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing Inc., Orlando, FL.

Reddy, K. R., D'Angelo, E. M., and DeBusk, T. A. 1990. Oxygen transport through aquatic macrophytes: The role in wastewater treatment. J. Environ. Qual. 19:261-267.

Reddy, K. R., Patrick, W. H., and Lindau, C. W. 1989. Nitrification-denitrification at the plant-root-sediment interface in wetlands. Limnol. Oceanogr. 34:1004-1013.

Reed S. C., and Brown, D. S. 1992. Constructed wetland design--The first generation. Water Environment Research. 64(6):776-781.

Reed S. C., Middlebrooks, J. E., and Crites, R. W. 1988. Natural Systems for Waste Management and Treatment. McGraw-Hill Book Company, New York.

Resh, H., 1981, Hydroponic Food Production: A Definitive Guidebook for the Advanced Homegrower and Commercial Hydroponic Grower. Woodbridge Press Company, Santa Barbara, California.

Rinzema, A., and Lettinga, G. 1985. Anaerobic treatment of sulfate-containing wastewater. pp 66-102. In: Wise, D. L. (ed). Biotreatment Systems: Volume III, CRC Press Inc., Boca Raton FL.

Rittman, B. E., Crawford, L., Tuck, C. K., and Namkung, E. 1986. In situ determination of kinetic parameters for biofilms: Isolation and characterization of oligotrophic biofilms. Biotechnology and Bioengineering. 28:1753-1760.

Robertson, L. A., and Kuenen, J. G. 1983. Aerobic denitrification: A controversy revived. Arch. Microbiol. 139:351-354.

Rogers, K. H., Breen, P. F., and Chick, A. J. 1991. Nitrogen removal in experimental wetland treatment systems: Evidence for the role of aquatic plants. Water Environment Research. 63(7):934-941.

SAS. 1987. Statistical Analysis Systems 6.04. SAS Institute. Cary, North Carolina.

Seidel, K. 1976. Macrophytes and water purification. In: Toubier, J. and Pierson, R. W. Jr. Biological control of Water Pollution, University of Pennsylvania Press, Inc., Philadelphia, PA. p 109-121.

Smart, P. L., and Laidlaw, I. M. S. 1977. An evaluation of some fluorescent dyes for water tracing. Water Resources Research. 13:15-33.

Smith, M., and Rees, T. 1979. Pathways of carbohydrate fermentation in the roots of marsh plants. Planta. 146:327-334.

Snoeyink, V. L., and Jenkins, D. 1980. Water Chemistry. John Wiley and Sons, New York.

Stouthamer, A. D. 1988. Dissimilatory reduction of oxidized nitrogen compounds. pp 245-304. In: Zehnder, A. J. B. (ed). Biology of anaerobic Microorganisms. John Wiley and Sons, New York.

Stumm, W. 1966. Redox potential as an environmental parameter: Conceptual significance and operational limitation. pp 1-16. In: Third International Conference on Water Pollution Research. Water Pollution Control Federation. Washington D.C.

Stumm, W., and J. J. Morgan. 1981. Aquatic Chemistry. 2nd ed. Wiley-Interscience, New York.

Szpyrkowicz, L., Rigoni-Stern, S., and Zilio-Grandi, F. 1991. Nitrification and denitrification of tannery wastewaters. Water Research WATRAG. 25:1351-1356.

Takai, Y., and Kamura, T. 1966. The mechanism of reduction in waterlogged paddy soils. Folia. Microbiol. 11:304-311.

Taylor, G. J., Crowder, A. A., and Rodden, R. 1984. Formation and morphology of an iron plaque on the roots of Typha latifolia L. grown in solution culture. Amer. J. Bot. 71:666-675.

Teal, J. M., and Kanwisher, J. 1961. Gas exchange in a Georgia salt marsh. Limnol. Oceanogr. 6:388-399.

Teal, J. M., and Peterson, S. B. 1993. A solar aquatic system septage treatment system. Environ. Sci. Technol. 27(1):34-37.

Tiedje, J. M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. pp 179-244. In: Zehnder, A. J. B. (ed), Biology of Anaerobic Microorganisms. John Wiley and Sons, New York.

USEPA 1983. Methods for Chemical Analysis of Water and Wastes. Environmental Monitoring and Support Laboratory, United States Environmental Protection Agency, Cincinnati, Ohio.

USEPA 1988. Design Manual: Constructed Wetlands and Aquatic Plant Systems for Municipal wastewater Treatment. United State Environmental Protection Agency. U.S. EPA. EPA/625/1-88/022

Vershinin, A. V., and Razanov, A. G. 1983. The platinum electrode as an indicator of redox environment in marine sediments. Mar. Chem. 14:1-15.

Water Pollution Control Federation (WPCF). 1983 Nutrient Control:Manual of Practice FD-7 Facilities Design. Water Pollution Control Federation. Washington, D.C.

Water Pollution Control Federation (WPCF). 1988 O & M of Trickling filters, RBCs, and Related Processes: Manual of Practice OM-10. Water Pollution Control Federation. Washington, D.C.

Water Pollution Control Federation (WPCF). 1990. Natural Systems for Wastewater Treatment: Manual of Practice No. FD-16, Water Pollution Control Federation. Alexandria, Virginia.

Whipps, J. M., 1990. Carbon economy. pp 59-98. In: Lynch, J. M. (ed). The Rhizosphere. John Wiley and Sons, New York.

Whitfield, M. 1969. Eh as an operational parameter in estuarine studies. Limnol. Oceanogr. 14:547-558.

Whitfield, M. 1974. Thermodynamic limitations on the use of the platinum electrode in Eh measurements. Limnol. and Oceanogr. 19:857-865.

Widdel, F. 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. pp. 469-586. In: Zehnder, A. J. B. (ed), Biology of Anaerobic Microorganisms. John Wiley and Sons, New York.

Widdel, F., and Pfennig, N. 1984. Dissimilatory sulfate- or sulfur-reducing bacteria. In: Bergey's Manual for Systematic Bacteriology. Williams and Wilkins, Baltimore.

Wolverton, B. C., McDonald, R. C., and Duffer, W. R. 1983. Microorganisms and higher plants for wastewater treatment. J. Environ. Qual. 12:266-242.

Wolverton, B. C. 1984. Upgrading septic tanks using microbial/plants filters. J. Mississippi Academy of Sciences. 24:19-25

Wrenn, B. A. 1992. Substrate interactions during anaerobic biodegradation of 1,1,1 trichloroethane. Ph.D dissertation, University of Illinois, Urbana-Champaign.

Yoda, M., Mikio, K., and Yusho, M. 1987. Long term competition between sulfate-reducing and methane-producing bacteria and acetate in anaerobic biofilm. Water Res. 21:1547-1556.

Yoshida, Y., and Hirata, K. 1981. Studies on the high rate removal of nitrogen with intermittent aeration III: Bacterial flora in treatment tank from which nitrogen was removed by intermittent aeration. Nippon Suisan Gakkaishi, 47:1205-1208.

Young, J. C., and McCarty P. L. 1969. The anaerobic gravel filter for waste treatment. J. Water Pollut. Contrl. Fed. 41:R160-R173.

Zaiss, U. 1984. Acetate, a key intermediate in the metabolism of anaerobic sediments containing sulfate. Arch. Hydrobiol. Beih. 19:215-221.

Zehnder, A. J. B., and Koch, M. E. 1983. Thermodynamic and kinetic interactions of the final steps in anaerobic digestion. In: Van der Brink, W. J. (ed). Proceedings of European Symposium on Anaerobic Waste Water Treatment, The Hague, Netherlands.

Zehnder, A. J. B., and Stumm, W. 1988. Geochemistry and biogeochemistry in anaerobic habitats. pp 1-38. In: Zehnder, A. J. B. (ed), Biology of Anaerobic Microorganisms. John Wiley and Sons, New York.

BIOGRAPHICAL SKETCH

Peter Smiley Burgoon arrived in this world on December 9, 1956, eight minutes after his fraternal twin Thomas, and two minutes before his identical twin Michael. A short sigh of relief was heard from their blessed mother at the immedaite loss of a 15-pound burden; this was short lived however, as they developed into hundreds of pounds of trouble. They grew up exploring the rural countryside of Pennsylvania with an older brother and four sisters.

Peter received a Bachelor of Science degree in Marine Biology from Long Island University-Southampton College in May 1979. The next four years, he conducted research on integrated hydroponic aquaculture systems at The New Alchemy Institute in Falmouth, Massachusetts. His interaction with other scientists and engineers at the Institute helped establish his faith in science as a valuable tool for understanding, creating, and maintaining, a hospitable environment for all inhabitants on Earth.

This led to a Masters of Engineering in the Department of Environmental Engineering Sciences at the University of Florida in May 1989. In the future, Peter looks forward to joining his peers and cronies in research, teaching, and consulting on methods to clean the soil and water.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

K. Ramesh Reddy

Konda R. Reddy, Chair
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P. Suresh C. Rao
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Graetz

Donald A. Graetz
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Koopman
Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John Zoltek Jr.
Professor of Environmental Engineering Sciences

This dissertation was submitted to the Graduate Faculty of the College of Education and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1993

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School